COMMONWEALTH EDUCATION AND DEVELOPMENT

IN an adjournment debate on aid to under-developed territories on December 17, Mr. A. E. Cooper said that technical assistance is one of the greatest contributions that Great Britain can make, possibly exceeding in importance what we can do in the way of finance. He referred particularly to the assistance we can give in agriculture, and suggested that we should be more concerned as to the manner in which such technical assistance can be given and the right type of technical colleges provided. Mr. Cooper also believes that there are too many organizations through which assistance can be given, and that the Colombo Plan or similar regional organizations provide the best basis and are more effective than the United Nations. Mr. J. Hall, who stressed also the bearing of a healthy balance of payments on the amount of assistance Britain can give, likewise suggested that our most important contribution is in the field of technical assistance. Here the real difficulty is the supply of technicians and skilled labour available, and he believes that the Government could do more to encourage the training of technicians for service overseas, particularly on Government service. The provision of sufficient qualified teachers, too, is a major difficulty in establishing schools, technical colleges and universities overseas, particularly while service overseas does not rank for pension, and Mr. Hall thinks that the Government could help here by making it possible for such service to count for pension when teachers returned to teach in Britain. Sir Edward Boyle, Financial Secretary to the Treasury, in welcoming the debate, commented that more interest is being taken in Britain and other Western countries in helping the less developed territories than ever before. Expenditure from United Kingdom public funds on economic and technical assistance to overseas countries and territories has risen from about £75 million in 1957–58 to about £100 million in 1958–59, and is likely to reach £150 million in the current financial year, exclusive of military assistance, emergency expenditure, relief measures and such like, which amount to roughly £30 million a year, while it is estimated that £300 million a year gross of private investment overseas, about half goes to the less-developed countries. Our bilateral assistance goes mainly and normally to Commonwealth countries: contributions to multilateral organizations go in support of their programmes of assistance to member countries. He believes that there is scope for building up the international institutions in this field, and Sir Edward referred in particular to the Colombo Plan, the International Monetary Fund, the International Finance Corporation and the International Development Association, to the last of which, if acceptable plans come to fruition, the United Kingdom will be subscribing about £50 million. Sir Edward welcomed the reference to agriculture and said that it would be impossible to over-rate the importance to the West of the success of India's second Five Year Plan.

This debate followed a discussion on the same subject initiated by the Marquess of Reading in the House of Lords on December 10 which, however, was concerned chiefly with the Colombo Plan and the countries of South and South-East Asia. Lord Reading himself discussed especially the question of teachers, as well as the importance of greater efforts for more adequate and widespread teaching of English in these countries; he also stressed the importance of the quality of the people who are sent out to render technical and other assistance. Lord Lansdowne, the Joint Parliamentary Under-Secretary of State for Foreign Affairs, just returned from the Colombo Plan Conference in Indonesia, referring particularly to technical assistance, said that up to June 1959 the United Kingdom had provided 2,600 training places in Britain for Colombo Plan students, and 360 United Kingdom experts had taken up assignments in Asia. Moreover, there are now in British universities and training colleges some 40,000 students from overseas, and we have already committed ourselves to £150 million under the Colombo Plan, of which nearly £30 million was spent in the year ended June 1959. Like other speakers in the debate, Lord Lansdowne referred to the need for more generous treatment of those who volunteered for teaching service overseas and for ensuring that their opportunities for promotion are not prejudiced thereby. The Earl of Lucan referred to the bearing on the problem of the new scholarships to be provided under the Commonwealth Scholarships Bill, and Lord Salter stressed the importance of careful choice of appropriate institutions, such as the World Bank, for the provision of capital assistance.

Lord Home, in replying on the debate, suggested that there is no field in which scientific and technical knowledge can pay higher dividends than that of agricultural and farming methods in the Asian countries. Referring to the improvement in recent years in international markets, he agreed on the necessity for stability in the prices of the primary products of Asian countries, and reminded the House that our ability to increase our aid must arise out of the improvement in our balance of payments. It is because of the increase in reserves that Britain has been able to double its subscription to the International Bank, and to increase by 50 per cent its subscription to the International Monetary Fund, while simultaneously its contribution from public funds to economic and technical assistance rose from £75 million in 1957–58 to more than £100 million in 1958–59 and has probably increased by a corresponding amount during the past twelve months. Lord Home agreed as to the importance of technical

assistance and of appropriate arrangements being made by local authorities and other employers of teachers to ensure that teachers do not suffer in their careers on return to Britain from service overseas. He believes that, for the time being, regional bodies offer the best means of dealing with the problem, and that the time is ripe for a concerted effort in Western Europe to raise the standard of living in the under-developed countries.

In spite, however, of the reference to the Commonwealth Scholarship Bill, there was no reference in either debate to the Commonwealth Education Conference, although the report of the Conference* dealt specifically with the training of teachers, the supply of teachers for service in countries of the Commonwealth other than their own and to technical education, and specific recommendations are made in the report to deal with these problems. Because the Commonwealth is a new experiment in human relationship, founded on a belief in the worth and dignity of the human individual and a recognition of the value of freedom and co-operative action, education is fundamental to the strength and stability of the Commonwealth, and to social justice and human dignity which must be its inspiration. There is an obligation on those with more highly developed educational facilities to help their fellow members.

The expansion of educational systems in all the countries of the Commonwealth requires new teachers on an unprecedented scale, and all countries, whatever their stage of educational development, need to expand and improve their facilities for teacher training. While some awards made under the Commonwealth Scholarship and Fellowship Plan are likely to be in the field of teacher training, in the long term the training of teachers on the scale required to meet normal needs must be achieved by the efforts of the countries themselves. The Conference Committee which dealt with this subject recommended that the teacher-training assistance provided should be carefully directed to secure the greatest possible benefit in proportion to the resources employed, and that some of the additional places now offered should be reserved for advanced or supplementary courses and only a relatively small proportion used exceptionally for initial training. Some of the funds should be used to enable teacher-training staff in the more advanced countries to assist the development of teacher-training in other countries.

Provision to give experience in training institutions to teacher-training staff from countries with less well-established systems of education is also recommended, and some members of teacher-training staffs in colleges receiving Commonwealth students from other countries should be enabled to visit and gain experience of the countries from which these students are drawn. To overcome the widespread shortage of candidates sufficiently equipped both technically and academically for immediate training as technical teachers, the Committee suggests the provision, where necessary by special courses, of a technical background for young people who have an adequate standard of general education. As a development of major importance, it also recommends that a proportion of the places to be made available should be allocated to training in the teaching of English as a second or later language.

The report stresses the importance of arrangements being made to receive students suitably on arrival and to induct them into their new surroundings and way of life, and of efforts, both before and after their arrival in the host country, to determine whether they need any special instruction in the language of their host country. Countries benefiting from the assistance offered by the United Kingdom should normally bear the cost of travel of their students, but a certain sum should be allocated from United Kingdom funds to assist the travel of students from relatively small and isolated territories. Adequate maintenance allowances are regarded as an integral feature of assistance towards training, and the cost of maintenance should be fully met from any grants made, even if this involves reducing the number of places originally offered by the United Kingdom.

A further Committee of the Commonwealth Education Conference considered the extent to which the countries of the Commonwealth could help each other to meet their needs for training teachers and staff who will train teachers, and the arrangements for providing such assistance. The numbers of primary school teachers required cannot be estimated with accuracy, but clearly many thousands will be required during the next four or five years, mainly from the countries concerned; but to assist these countries to train this large number, there is an immediate need for some 400 or 500 posts to be filled in their teacher-training institutions. Well over 1,000 teachers a year are needed for secondary schools, and about 200 teachers a year for technical schools, while universities throughout the Commonwealth are in need of staff, often in highly specialized subjects. The greatest needs are in Africa.

Already more than 2,500 teachers a year leave the United Kingdom for service in other Commonwealth countries and the Government is to make every effort to increase this number. Canada, Australia and New Zealand made definite offers of assistance at the Conference, and both India and Pakistan hope to encourage their teachers to serve in other Commonwealth countries. In its report the Committee recommended that, to ensure that assistance should be applied at the most fruitful and effective points, governments should concentrate on filling what might be described as key posts in countries where there is a shortage of teachers and that, where appropriate, the filling of such key posts should be facilitated by special allowances to teachers, assessed for the individual appointment. The interests of teachers serving abroad should be safeguarded in such matters as housing, children's education, adequate recognition of teaching service abroad, promotion prospects, re-employment and secondment rights, superannuation and re-settlement on return. The Committee

* Commonwealth Relations Office. Report of the Commonwealth Education Conference. Pp. 65. (Cmnd. 841.) (London: H.M. Stationery Office, 1959.) 4s. net.

also recommended that the question of establishing a Commonwealth Education Service should be examined, and that a small organization should be established to provide information about vacancies and the availability of staff for service abroad. Steps should also be taken to promote a climate of opinion which will recognize service abroad as a professional asset, and consideration should be given to the secondment of teachers between Commonwealth countries or between regional authorities in one country and another.

The Committee which considered the extent to which the countries of the Commonwealth could help each other to meet their needs for developing education and training in technical subjects, including training in industry, and the arrangements for providing such assistance, recognized that the training of technologists and technicians in adequate numbers is a serious problem, but pointed to some measures which could be taken immediately. A substantial number of additional places for Commonwealth students will be made available in the universities and technical colleges of the United Kingdom and Canada, and India and Pakistan are willing to offer similar facilities. The Committee strongly recommended that the interchange of scientists and technologists, which is of lasting benefit, should be encouraged and that all countries offering scholarships, other awards or facilities for education, training or research should devote a good proportion of the awards or facilities to technical education. More places should be provided in universities and higher technological institutes in Commonwealth countries for students from small countries which have no such facilities, and the governments of small Commonwealth countries should consider the co-operative establishment of regional technical teacher-training colleges. Postgraduate scholars in science and technology should be encouraged to take part-time teacher-training courses, and the Committee also directs attention to the need for close and continuous attention to the supply of books and periodicals, as well as to the value of consultation with the appropriate professional bodies regarding qualifications awarded by technical institutions so as to ensure a high and uniform standard of attainment.

Normally, arrangements for mutual aid in technical education should be made bilaterally between the countries concerned, and every country should set up a permanent organization to supervise the detailed operation of mutual assistance; but a small information centre established in London might provide information about facilities for technical education and sources of advice available in the Commonwealth. Finally, the Committee recommends that efforts be made to increase mutual assistance by providing opportunities for training and experience in industry from people from Commonwealth countries, and that technical education should receive a full share of all the various kinds of financial and other assistance resulting from the Conference.

The report of the Commonwealth Education Conference estimates that the arrangements agreed at the Conference will involve new expenditure of at least £10 million during the next five years, and it recommends the establishment of a committee in London composed of a representative of each member country of the Commonwealth, with appropriate arrangements to represent the interests of the Colonial territories and provided with a small administrative unit. For the rest, the strength of the new arrangements rests in men and women and the cultivation of personal relationships; and the administrative arrangements proposed are designed to assist the normal channels to flow more freely. Apart indeed from the stimulus given by the Conference to the flow of ideas and exchange of experience, this stimulus to the interchange of scholarship, either in science and technology or in other fields, is of as vital import to the effectiveness of the aid given by the West to the under-developed territories as is the stimulus given to education itself. Both alike should broaden the mutual understanding upon which effective aid must rest and remove suspicions and distrust. Now that the Western governments are seeking to co-ordinate their plans for economic aid to the under-developed countries, education assumes an increasing importance not simply in the matter of technical assistance but also in the establishment of institutions which will enable assistance, whether financial or economic or social, to be given efficiently and swiftly to the greatest effect.

MECHANICS IN THE MIDDLE AGES

The Science of Mechanics in the Middle Ages

By Marshall Clagett. (University of Wisconsin Publications in Medieval Science, Vol. 4.) Pp. xxix + 711. (Madison, Wisconsin: University of Wisconsin Press, 1959.) 8 dollars.

IT has long been customary to consider the Middle Ages as a period without any importance for the rise of modern natural science. The Greek contributions were duly studied; then came a blank of several centuries, commonly designated as the Dark Ages, and the story began again with Galileo, whose work seemed to arise out of nothing. The scholastic ideas on natural science were deemed wholly barren and, if ever mentioned, only to be ridiculed.

A marked change in this unfruitful and essentially unhistorical approach was brought about at the beginning of this century by Pierre Duhem, French historian of science, who in a series of brilliant works assumed an entirely new attitude towards the scholastic ideas on natural science in general and on mechanics in particular. He showed that several important notions and results, which always had been considered as typical of the revival of learning in the 16th and 17th centuries, had to be dated back some two or three centuries. His investigations were continued in Europe by C. Michalsky, Anneliese Maier and others. At the same time there arose in the United States a new centre of medieval studies at the University of Wisconsin. There Marshall Clagett notably distinguished himself by penetrating studies in the field of medieval mechanics. The book mentioned above, together with an earlier volume on medieval statics published some years ago by him in

collaboration with Ernest Moody ("The Medieval Science of Weights", Madison, Wisconsin, 1952), may be considered (except for new discoveries) the definitive exposition of our present knowledge of the subject. The huge volume is divided into four parts, the first of which contains the essential contents of the former volume with some important supplements. Parts II and III are devoted to kinematics and dynamics respectively and Part IV is an extremely useful summary of the whole subject of medieval mechanics. After an introduction the author generally gives an English translation of the documents at issue, followed by a lucid commentary and by the original text.

The most important subjects treated are: (1) the work of Gerard of Brussels, the importance of which was appreciated some years ago by the author and discussed by him in vol. 12 of *Osiris* (73–175; 1956); (2) the contribution of the scholars of Merton College, Oxford, especially Thomas Bradwardine, William Heytesbury, Richard Swineshead and John of Dumbleton, towards the kinematics of uniformly accelerated motion; (3) the application of plane geometry to kinematics in the graphical method, resulting in a geometrical proof of the so-called Mertonian rule for uniformly accelerated motion, formerly called Oresme's rule by Duhem; (4) Bradwardine's dynamic law of movement, which, though not valid, contributed essentially to the rise of mathematical physics; (5) the theory of impetus and its application in the explanation of the acceleration of free fall and of the movement of celestial bodies; (6) the daily rotation of the Earth, as discussed by Buridan and Oresme, who both advanced strong arguments in favour of it, without, however, bringing themselves to accepting it.

The way in which all these subjects are treated by the author deserves our admiration and assures him a prominent place among the authorities on medieval mechanics. E. J. DIJKSTERHUIS

PHYSIOLOGY OF REPRODUCTION

Reproduction in Domestic Animals

Vol. 2. Edited by H. H. Cole and P. T. Cupps. Pp. xi+451. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 13 dollars.

FORMERLY the physiology of reproduction was studied mainly in small laboratory animals, but, with the increasing economic importance of the larger domestic animals, during the past two decades much work has been done in this field. The second volume of the book on "Reproduction in Domestic Animals" emphasizes the differences which exist between species in the details of their reproductive process.

Six of the eleven chapters, each written by an expert on the subject, deal with the male: spermatogenesis by Ortavent, biochemistry by Mann, libido and storage of semen by Rowson, insemination by Almquist, and the fowl by Lorenz. These present a most comprehensive review of recent literature on the subject. Among many interesting subjects dealt with are calculations of the daily sperm production, species differences in semen characteristics, factors affecting libido and semen quality, the deep-freezing of semen, differences in the technique of insemination in different species, and the effects of œstrogens on the cock. Among the other chapters, one on nutrition by Moustgaard is an important contribution, for among other things it gives a very good account of the nutrient requirements for fœtal development in different species, a subject which has hitherto received but little attention. The effects of underfeeding of the various food constituents, including vitamins and minerals, on the development and viability of the offspring as well as on the functions of the reproductive organs are also described.

In another chapter, by Clegg and Ganong, other environmental factors affecting reproduction in different species are outlined. These include the effects of variation in daylight hours, temperature and humidity together with 'social stimuli', such as that of the sexual display in birds. The physiology of the female fowl is described by Van Tienhoven, and this includes sections dealing with behaviour in mating and broodiness; the effects of different hormones are also reviewed. A chapter on anatomical and physiological factors affecting fertility by Mixner deals with intersexuality and such things as testicular and ovarian hypoplasia, hernias, cystic ovaries, and embryonic mortality. Hart and Osebold describe the infectious diseases of bacterial, virus and protozoan origin.

The book should be most useful to agriculturists and veterinarians, as well as to those engaged in human medicine, for many of the experimental results reviewed are fundamental to the physiology of mammalian reproduction. J. HAMMOND

HISTORY OF BIOLOGY

A History of Biology to about the Year 1900

A General Introduction to the Study of Living Things. By Dr. Charles Singer. Third and revised edition. Pp. xxxvi+580. (London and New York: Abelard-Schuman, Ltd., 1959.) 50*s*. net.

WHEN writing or talking about biology there are two schools of thought, namely the one which maintains that to try to learn or understand the history of the subject is a waste of time, and the other which holds that to understand any subject one must appreciate and know its beginnings and its development. These two differing attitudes can be reconciled by the assumption that biology only found itself as a science about 1850 and what went before is 'irrelevant and immaterial'.

To this reconciliatory suggestion Dr. Singer has produced a stimulating reply in the third and revised edition of "A History of Biology", which is based on the thesis that "the man of science must work backwards for a space . . . for unless we adopt a wide historical outlook we shall find ourselves unable to grasp the nature of the great problems which we are discussing".

After an introduction in which the claims of the historical approach are advanced, the book is divided into three parts. The first part, entitled "The Older Biology", treats aspects of the subject from Hippocrates to Harvey and the circulation of the blood in 1628. In the second section, the "Historical Foundations of Modern Biology" are laid by the development of experimental method in the works of Bacon and Descartes, and followed through the ideas of taxonomy and evolution into the immediate post-Darwin era. There is a number of topics of general interest in the third section such as "Cell and Organ-

ism", "Development of the Individual", etc., each of which is examined from a historical point of view.

It is therefore an ambitious book, but as an introduction to the study of living things (which is the sub-title) it seems to me to fall a bit short of the mark. For example, Dr. Singer has chosen his end-point at about 1900. This is sixty years ago, and since that time biology has progressed so quickly and so far that the average reader and television viewer will wonder where he can find the links between this book and the science he sees and hears about him every day. It is realized that to write an up-to-the-minute history of biology is beyond the scope of any man, but when the first edition of this book was published in 1931 it was only thirty years out-of-date, whereas, although additions have been made, it is now sixty years behind the times.

It is proper that a 'history' should contain the names of many people, places and things. But it is bewildering to someone seeking an introduction to find so many things, dates and people on each page and my reaction was to begin to skip certain paragraphs as contributing too little to the main theme.

Nevertheless this book is not simply a compendium and it clearly is a work of scholarship and insight. I found it interesting, stimulating, and in places almost exciting. It is well written and illustrated with much to give to both professional and amateur biologists, and if it has its faults they spring more from the dual task which Dr. Singer has set himself than from any deficiencies in style or material.

A. R. GEMMELL

MYCENÆAN AND CLASSICAL GREECE

The Annual of the British School at Athens

No. 52, 1957. Pp. viii+264+50 plates. (London: The British School at Athens, 1959.) 63*s*. net.

THIS is an interesting number. J. Boardman writes on "Early Euboean Pottery and History", and re-opens the question whether the "Chalcidian" ware of the sixth century B.C., assigned by fashionable archæologists to Italiote workshops, was in fact made in Chalcis after all. Euboic letter-forms show, he thinks, close contact between Euboea and Phoenicia in the eighth century B.C. The notorious early war between Chalcis and Eretria for the Lelantine Plain is dated by most scholars around 700 B.C., and supposed to have ended in the triumph of Chalcis. Boardman points out, however, that possession of the plain, necessary to Chalcis, matters little to Eretria, and that Eretria apparently suffered no material setbacks between 800 and 600. I wonder whether one can go farther, and put the end of the war, the Chalcidian victory and the rise of Chalcidian ware all well into the sixth century. This is a very stimulating paper.

A. Andrewes considers early Hellenistic lists of *patrai* in Kamiros. At this date, they were subdivisions of local tribes, and represented a complicated modification of the earlier gentile *patrai*.

V. Karageorghis identifies a Mycenæan vase-painter working in the Levant, who favoured chalices and bowls among shapes, and animal protomae among ornaments.

R. A. Higgins re-examines the Aegina Treasure, now in the British Museum, which recent scholars have dated at about 800 B.C. He demonstrates that, however it reached Aegina, it is mostly, perhaps entirely, Middle Minoan, of about the seventeenth century, and close in style to the treasure of Mallia. His arguments seem conclusive and important.

G. E. Bean and J. M. Cook continue their survey of the Carian Coasts. They retail evidence for their view that classical Cnidus was at Burgaz, near Datça, and that the classical Triopion, at Kumyer near by, had an enormous sea-wall. They also examine the large walled city of Theangela, in their view a creation of Mausolus. But they attribute the large landward wall of Iasus to the late-fifth-century Persian rebel, Amorges, the ally of Athens. Moreover, they believe that at Meropis, on Cos, the Athenians set up their own copy, in Attic lettering, of the famous monetary decree of about 449 B.C., because they had an Athenian station there. The argument is interesting, although we could not have inferred from the decree that coins with the legends "KOION" and "KOS" would soon afterwards be struck at Meropis. Indeed, the wording of the decree seems to give a monopoly of striking to the mint at Athens. This article sturdily and refreshingly rebuts various pedantic dogmas of Louis Robert.

T. E. Jones, L. H. Sackett and C. W. T. Eliot survey the Děma, the famous westward-facing wall between the Athenian and Thriasian Plains, and conclude that it dates from the years of panic after Chaeronea (338 B.C.). They note that its plan uses the "indented trace", here closely resembling the landward wall of Iasus, which they date (p. 182) to the fourth century. They were unaware, of course, of Cook and Bean; and it is amusing to see these various dates put forward in one annual.

The number also includes some stray linear *B* tablets from Cnossus, published by John Chadwick, the last harvest of Wace's campaigns at Mycenae, R. Hope Simpson's arguments for a Mycenæan Messene and Sinclair Hood's account of a tower and wall of *c*. 300 B.C. near the Zafer Papoura cemetery at Cnossus.

HUGH PLOMMER

BIOCHEMISTRY OF HUMAN GENETICS

Human Biochemical Genetics

By Dr. H. Harris. Pp. viii+310. (Cambridge: At the University Press, 1959.) 37*s*. 6*d*. net.

NO subject in biology is of greater current interest than genetics, and the genetics of human beings is becoming of immense practical importance. It is ironical that most of our knowledge of human genetics comes from a very few fields—in particular blood groups and biochemistry. Several excellent treatises on blood groups are available, but this book by Dr. Harris is the first comprehensive text on the latter field.

In his introduction Prof. L. S. Penrose rightly remarks that the publication of A. E. Garrod's classic "Inborn Errors of Metabolism" marked the beginning of a revolution in genetics. Yet its fruits are slow to ripen. From the last edition of Garrod's book in 1923 until Dr. Harris published his previous work "Introduction to Human Biochemical Genetics", twenty years passed, during which time very little progress was made in human biochemical genetics. Since then a flood of new and fascinating work has

appeared—for example, that on the glycogen storage diseases, galactosæmia and the chemical differences between the hæmoglobin variants. The present volume is thus much more than a new edition of Dr. Harris's previous book. It is a good deal longer and well up to date. The chapters deal in succession with a historical sketch, simple genetics, the metabolism of amino-acids and of carbohydrates, the abnormal hæmoglobins, the blood groups, plasma proteins, miscellaneous conditions and the problem of gene action. The last chapter is in many ways the weakest, though it is undoubtedly the most difficult subject to handle. The other chapters are all of an even and high standard. In matters of fact the book is remarkably accurate; in matters of interpretation it is lucid and well balanced.

The author has somewhat cramped his style by omitting most of the subjects which are speculative, poorly understood, or which cannot be given a firm chemical basis. For example, little mention is made of favism, or of the ability to taste phenylthiocarbamide, or to smell hydrogen cyanide. Population genetics is only briefly treated. This gives the reader the impression of a neatly ordered but restricted field, and the book is therefore less stimulating than it might have been if some of the more provocative themes had been included.

One of the most encouraging aspects of the work described here is the remarkable possibility of cure of the symptoms of some of the inherited diseases. This emphasizes the point that whatever the eugenic problems of our remote descendants, the challenge of our own time is primarily a therapeutic one.

It is a pleasure to handle a book which is so well printed and well bound. The format is the Cambridge University Press's best—well-spaced type on good paper, with contrasting founts for genetic symbols, and clear uncramped figures. The index is reasonably full. It is very well worth the price, and should be in the library of every department of genetics.

P. H. A. Sneath

COTTON AND GENETICS

The Application of Genetics to Cotton Improvement

By Sir Joseph Hutchinson. Pp. viii + 87. (Cambridge: At the University Press, 1959. Published in association with the Empire Cotton Growing Corporation.) 15*s*. net.

THIS slim volume of eighty-six pages is based on a number of lectures given by Sir Joseph Hutchinson at the North Carolina State College, with some additions from recent work at the Namulonge Station, of which he was director.

The comprehensive title of the book is scarcely applicable to the restricted range of topics discussed. The reader is led to expect a scholarly appraisal of the methods employed and results obtained in other countries and by other workers. He will be disappointed.

The work is divided into six sections, which deal respectively with cotton relatives, the origin and spread of the Old World cottons, the origin and spread of New World cottons, the pattern of evolution in cotton-breeding systems, and the improvement of African cottons.

Discussion of problems implied by the title has to be confined within the limits of forty pages. This must be the author's excuse for a mode of treatment lacking in historical perspective, and which promulgates numerous one-sided opinions on questions still acutely controversial.

Much space is given to a discussion of the theory of the recent origin of the New World tetraploid cottons put forward by the author and his colleagues, R. A. Silow and S. G. Stephens. When first put forward in 1947, this theory was greeted with almost unanimous incredulity. The author now summarizes recent cytological and archæological evidence which at last consigns the theory to respectable burial, although he does not formally reject it himself.

The work of Manning and Sir Joseph Hutchinson on the response of Uganda cotton to selection is described. It is shown that by using a selection index in relatively inbred material, a yield advance of the order of 4 per cent per annum was achieved over eight generations of selection.

This technique is important enough to find a place in cotton breeding, but will probably not come into general use unless it can be combined with the mass pedigree system, and with the recent extensions and modifications of this used by Knight in the Sudan. These comprise selfing alternated with panmixis; transfer of genes, and transfer of gene complexes by alternate generations of back crossing and selfing.

Nowhere is it stated that the increase in yield obtained in Uganda was in a cotton with the minute yield of 1,000 lb. of seed cotton per acre—approximately one-third of that currently obtained in more favoured countries.

The references to breeding policy in Egypt are unfair and misleading. The brilliant work of Balls and his colleagues in Egypt in almost every branch of science bearing on both yield and quality has been a source of inspiration to cotton workers for nearly a half-century. To omit the name of the inventor of cotton technology is inexcusable; to misrepresent his policy is both mischievous and irresponsible.

It is clear that Balls was never unmindful of either yield or quality. He has written books on both subjects. He tried to strike a balance between the two; he "bred to dollars". Although it receives no mention, the work of Hancock, who subjected hundreds of progeny samples to a small-scale spinning test both for the direct evaluation of quality and for an assessment of genetical purity, is of far-reaching importance since it concerns the very future of cotton as an agricultural crop.

Peruvian workers will note with raised eyebrows the statement that Peruvian Tanguis is "fairly primitive by the standard of most cotton growing countries, being a perennial". In point of fact Tanguis has a low node number, and is no more a perennial than Sea Island, Upland or Egyptian cotton. Far from being primitive it is a highly selected and productive cotton much appreciated on the world market.

Apart from these criticisms, and others for which space is lacking, the book contains much valuable information on the development and evolution of cultivated cottons. It is authoritative when the author refers to material well known to him, and it brings up to date the account given by him and his colleagues in their well-known work "The Evolution of *Gossypium*".

S. C. Harland

POTASSIUM–ARGON AND RUBIDIUM–STRONTIUM AGES OF SOME GRANITES FROM BRITAIN AND EIRE

By Prof. J. L. KULP, Dr. L. E. LONG and C. E. GIFFIN
Geochemical Laboratory, Lamont Geological Observatory, Columbia University, Palisades, New York

Dr. A. A. MILLS*, Dr. R. StJ. LAMBERT and Dr. B. J. GILETTI
Department of Geology, University Museum, Oxford

AND

Dr. R. K. WEBSTER
Analytical Chemistry Branch, Atomic Energy Research Establishment, Harwell, Berkshire, England

THE development of modern techniques of isotopic age determination makes possible an absolute definition of the geological time-scale. The scale in common use was constructed by Holmes[1,2] and placed the beginning of the Upper Cambrian at 450 million years ago. He was handicapped, however, by uncertainties in the age and stratigraphic assignment of the samples then available; and subsequent investigators[3–9] and Holmes himself (personal communication) have found it necessary to suggest that the scale should be lengthened.

The most radical revision was that of Mayne *et al.*[8], who suggested that the age of the Upper Cambrian should be nearer 650 million years. This time-scale has already been criticized on general grounds[10,11], but in view of the importance of the revision it was also considered desirable to check some of their results. In an earlier paper[10] it was concluded that the evidence for this extended time-scale rests largely with the measurements made by Mayne *et al.*[8] on British granites. The present article gives some new data obtained for the same rock units by using the isotope dilution technique for argon determinations, and by making independent age determinations by the rubidium–strontium method.

The work at Oxford of Mayne *et al.*[8] began as an investigation, under the direction of Prof. L. R. Wager, into the ages of British granites and related intrusions. In the British Isles, two major sets of granitic intrusions have unusually well-defined stratigraphic relationships connected with the Caledonian and Hercynian earth movements. No subsequent alteration of these intrusions has occurred, with the exception of late-stage magmatic effects[12,13]. The ages obtained by the potassium–argon and rubidium–strontium measurements on the micas of these intrusions should therefore reflect the actual date of intrusion and crystallization of these magmatic bodies.

Among the Caledonian intrusions are the 'granite' masses of Shap, Leinster and Creetown (the latter listed by Mayne *et al.*[8] as "Cairnsmore of Fleet"; the specimens analysed come from a large granite dyke at Creetown which is probably related to the Cairnsmore of Fleet intrusive) which all cut a folded Ordovician–Silurian series. This series is overlain by Upper Devonian–Lower Carboniferous sediments, which were deposited on the folded and eroded Lower Palæozoic strata.

The adamellite of Shap cuts Ordovician strata; but the metamorphic aureole surrounding the Shap affects Upper Silurian (Ludlovian) strata, which are the highest beds involved in the pre-intrusion folding[14]. The basal Carboniferous nearby, which is of Lower Dinantian age, contains Shap pebbles[15]. The age of the intrusion is thus post-Ludlovian and pre-Carboniferous. Further, it is probable that it should be assigned to the Downtonian (the transitional Siluro–Devonian stage) or the Lower Devonian on account of general petrological and structural evidence, which, however, is inconclusive.

In Leinster, the upper limit of age is provided by Ordovician deposits and the lower by Upper Devonian. The Creetown granite cuts Upper Silurian strata; but no lower age limit is definable. In both cases it is generally assumed from petrological similarities that these masses were intruded at the same period of igneous activity as the Shap adamellite.

The Hercynian granites of Britain occur in Devon and Cornwall, and cut a folded Devono–Carboniferous series which is unconformably overlain by continental sediments conventionally referred to the Permian and Trias. The Dartmoor granite cuts a folded series containing Viséan, Namurian and Lower Westphalian fossils[16], though in the immediate neighbourhood of the granite only Viséan fossils are known[17]. Thus, while the Dartmoor granite is almost certainly post-Lower Westphalian, Lower Stephanian strata in Somerset are affected by the same fold system[18] and it is therefore probable that the granites are of latest Stephanian or Permian age. The Land's End granite is correlated with the Dartmoor granite on geochemical and petrological grounds.

The analytical results are given in Table 1 (potassium–argon determinations at Lamont) and Table 2 (rubidium–strontium determinations at Oxford and Harwell). The analyses were all made on samples of biotite separated at Oxford, except for the Leinster granite which was collected by one of us (J. L. K.), with the co-operation of Mr. M. V. O'Brien, director of the Geological Survey of Eire. Samples were further purified by the analysts separately, so that it is not possible to compare analytical details between laboratories. All potassium analyses, however, were made on carefully split aliquots of the final preparations used for the argon measurements.

The isotope dilution method using enriched argon-38 as 'spike' was used for all argon determinations. Over the period occupied by this work, five different spike sets were employed. Analyses of the Massachusetts Institute of Technology standard biotite by four spike sets are given in Table 3. These results agree with measurements reported by four other laboratories on this standard to within 1 per

* Present address: Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia.

Table 1. Potassium–Argon Ages
$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$; $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

Rock unit and location	Mineral	Specimen number Oxford	Specimen number Lamont	Sample wt. (gm.)	A-38 spike pressure (cm. Hg × 10^4)	A-40* (p.p.m.)	A-40* / Total A-40	Potassium (per cent)	Apparent age (m.y.) This study	Apparent age (m.y.) Mayne *et al.* (ref. 8)
Hercynian Granites										
Dartmoor granite	Biotite	20,503	1-*B*	2·388	21·0	0·148	0·66	(6·93)	279 ± 8	
Haytor Rocks,			3-*B*	2·083	5·19	0·132	0·94	6·57	263 ± 13	
Dartmoor, Devon			4-*B*	3·577	6·09	0·150	0·98	7·22	271 ± 8	
			4-*B*	3·072	6·09	0·148	0·91		269 ± 8	
								Weighted average	271 ± 5	305
Land's End granite	Biotite	9,437	1-*B*	1·883	5·19	0·134	0·90	7·17	250 ± 15	340
Lamorna Cove,										
Land's End,										
Cornwall										
Caledonian Granites										
Shap adamellite	Biotite	7,680	1-*B*	2·568	6·18	0·198	0·91	6·36	393 ± 12	
Shap,			1-*B*	2·505	5·19	0·196	0·97		391 ± 20	
Westmorland		20,405	2-*B*	2·870	6·09	0·185	0·90	5·89	397 ± 12	
			2-*B*	2·429	6·09	0·178	0·85		383 ± 12	
								Weighted average	391 ± 7	475–510
Creetown granite	Biotite	20,501	2-*B*	1·488	4·91	0·215	0·92	7·13	382 ± 11	470
Creetown,			2-*B*	1·528	4·91	0·219	0·96		390 ± 12	
Kirkcudbrightshire								Average	386 ± 8	
Leinster granite	Biotite	—	1-*B*	3·622	6·09	0·184	0·86	6·21	377 ± 11	
Dublin,			1-*B*	3·173	6·09	0·187	0·97		381 ± 11	
Eire	Muscovite	—	1-*M*	2·836	6·09	0·258	0·93	8·32	392 ± 12	
			1-*M*	2·450	6·09	0·258	0·95		392 ± 12	
								Weighted average	386 ± 6	—

* Indicates radiogenic component.
Parentheses indicate potassium analysis on identical material at Oxford, by flame photometer. All others by Ledoux and Co.

cent (P. M. Hurley, personal communication). The coefficient of variation of radiogenic argon measurements at Lamont, based on many duplicate analyses over the past two years, is less than 2 per cent. The absolute uncertainties for samples in this age-range are estimated to be ± 2 per cent, based on the performance of the spike calibration system and the results shown in Table 3. A somewhat higher error is assigned to the samples run with spike ($5{\cdot}19 \times 10^{-4}$ cm. mercury) as this was calibrated indirectly by runs on micas of known radiogenic argon content. The details of these experimental procedures are being given elsewhere[19].

The potassium analyses were performed (with one exception) by Ledoux and Co., Teaneck, New Jersey, using a flame photometric method which has been shown to be reproducible with a coefficient of variation of 1–2 per cent based on blind duplicates. Their results agree with those of the University of Minnesota Rock Analysis Laboratory and isotope dilution analyses at the Lamont Geochemical Laboratory to within 2 per cent. Thus it may be assumed that the individual argon-40/potassium-40 ratios have a coefficient of variation of 3 per cent in this work. Additional inter-laboratory calibrations up to mid-1957 are given by Baadsgaard *et al.*[20] which show a coefficient of variation of only 3–4 per cent on the argon-40/potassium-40 ratios among all contributing laboratories (including Lamont).

The Dartmoor sample 1-*B* was analysed for potassium in the Oxford laboratory by C. McInnes and is believed to be accurate to at least 2 per cent, based on inter-laboratory calibration standards. The argon analysis was done on the identical material. An insufficient quantity was available for analysis by Ledoux and Co.

The potassium–argon isotopic ages were calculated using the decay constants indicated in Table 1. The average age was computed by calculating weights for the individual determinations from their probable errors. The Land's End granite sample was so small that a larger uncertainty is necessarily assigned to the potassium–argon determination of this specimen; but the agreement with the Dartmoor ages is such that the intrusions could have been emplaced at about the same time. The Shap, Creetown and Leinster granites have the same age within the experimental errors. The biotite from the Leinster granite is intimately chloritized and may have suffered slight loss of argon, but the difference between the average muscovite and biotite ages can also be comprehended by experimental error.

Determinations of the age of these micas by the rubidium–strontium method have been made independently at Oxford and at Harwell (Table 2). Both laboratories employed isotope dilution techniques throughout, using enriched rubidium-87 and either strontium-84 or strontium-86 tracers supplied by the Atomic Energy Research Establishment, Harwell. The Harwell techniques have already been described[21]. At Oxford the rubidium and strontium contents, and strontium isotopic composition, of each specimen were determined on a Metropolitan-Vickers *MS* 5 solid-source mass spectrometer; analytical details will be discussed in a subsequent paper.

The absolute error in the radiogenic strontium-87/rubidium-87 ratios determined at Oxford is estimated at ± 3 per cent, based on the agreement with the Harwell results and with samples the ages of which were also determined at Lamont (by B. J. G.) and elsewhere. The errors quoted for the Harwell determinations were calculated from the spreads of the various duplicate analyses. A half-life of $(4{\cdot}7 \pm 0{\cdot}1) \times 10^{10}$ years is used for rubidium-87 [22]. The rubidium–strontium ages for the Caledonian (Shap and Creetown) granites agree quite closely with the potassium–argon data reported in Table 1, and again indicate that the two intrusions were contemporaneous. The

Table 2. RUBIDIUM–STRONTIUM AGES OF BIOTITES
$t_{1/2} = 4{\cdot}7 \times 10^{10}$ yr.

Rock unit and location	Specimen number	Rb (p.p.m.)	Normal Sr (p.p.m.)	Sr* (p.p.m.)	Apparent age (m.yr.)	Laboratory
Hercynian Granite Dartmoor granite Haytor Rocks, Dartmoor, Devon	20,503	1,310 1,298 1,303	10 25 10	1·46 1·54 1·52	267 ± 8 284 ± 8 279 ± 8	Oxford Oxford Oxford
				Average	277 ± 5	
Caledonian granites Creetown granite Creetown, Kirkcudbrightshire	20,501	555	24	0·85	365 ± 11	Oxford
Shap adamellite Shap, Westmorland	7,680	846 848	12	1·27	385 ± 11	Oxford
		766	36	1·19	380 ± 19	Harwell
		801	39	1·30		
		773	34	1·19	364 ± 24	Harwell
		793	34	1·20		
				Weighted average	381 ± 7	

* Indicates radiogenic component.

rubidium–strontium values are much closer to the present potassium–argon ages than to those of Mayne *et al.*[8].

Should the $5{\cdot}0 \times 10^{10}$ year half-life of rubidium-87 (commonly in use at present) be employed, the value found for the average age of these rocks would increase by 6 per cent, and would agree with potassium–argon dates reported in Table 1 to within 2 per cent. The error in the reported rubidium–strontium ages would have to be about 25 per cent for these to agree with those of Mayne *et al.*[8].

The Hercynian data from all sources do not differ as much as the Caledonian dates, but agreement between the potassium–argon and rubidium–strontium measurements reported here is particularly close. Agreement with the Mayne *et al.*[8] dates would necessitate a 10 per cent error in the rubidium–strontium ages given here.

The age data for the Dartmoor granite should be considered in relation to a concordant uraninite age of 290 ± 10 million years for the Cornwall mineralization[23].

On Holmes's Scale *B*[2] the Carboniferous–Permian boundary was 203 million years and the Siluro–Devonian boundary 313 million years. The measurements reported here thus support suggestions for an extension of that scale. A revised time-scale based in part on some of these measurements has been published elsewhere[24]. The discrepancy between the potassium–argon results reported here and those of Mayne *et al.*[8] (which are given in the last column of Table 1) poses an analytical problem which has not yet been solved.

This research has been supported at Lamont by the Research Division, U.S. Atomic Energy Commission, under Contract *AT*(30-1)-1669. Grants from the Department of Scientific and Industrial Research and the Colonial Development and Welfare Organization have supported the research at Oxford. Enriched isotopes for the rubidium–strontium work were supplied by the Atomic Energy Research Establishment, Harwell.

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Table 3. ARGON ANALYSES ON *B*-3203 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY STANDARD BIOTITE)

Sample weight (gm.)	A-40* / Total A-40	A-40* (p.p.m.)	A-38 spike pressure (cm. Hg × 10^4)
4·166	0·94	0·689	21·0
1·668	0·92	0·687	6·09
1·985	0·97	0·688	6·09
1·759	0·92	0·687	6·01
1·539	0·97	0·692	4·91

* Indicates radiogenic component.

[1] Holmes, A., "The Age of the Earth" (Benn, London, 1927).
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Rock unit and location	Mineral	Specimen number Oxford	Lamont	Sample wt. (gm.)	A-38 spike pressure (cm. Hg × 10^4)	A-40* (p.p.m.)	A-40* / Total A-40	Potassium (per cent)	Apparent age (m.y.) This study	Mayne *et al.* (ref. 8)
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		766	36	1·19	380 ± 19	Harwell
		801	39	1·30		
		773	34	1·19	364 ± 24	Harwell
		793	34	1·20		
				Weighted average	381 ± 7	

* Indicates radiogenic component.

rubidium–strontium values are much closer to the present potassium–argon ages than to those of Mayne *et al.*[8].

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Table 3. Argon Analyses on *B*-3203 (Massachusetts Institute of Technology Standard Biotite)

Sample weight (gm.)	A-40* / Total A-40	A-40* (p.p.m.)	A-38 spike pressure (cm. Hg × 10^4)
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RELATIONSHIP BETWEEN THE RATE OF FALL-OUT AND THE CONCENTRATION OF STRONTIUM-90 IN HUMAN DIET IN THE UNITED KINGDOM

By Dr. J. D. BURTON, G. M. MILBOURN and Dr. R. SCOTT RUSSELL

Agricultural Research Council Radiobiological Laboratory, Letcombe, Wantage, Berkshire, and Agricultural Research Council Field Station, Compton, Berkshire

IF the long-term consequences of strontium-90 in world-wide fall-out are to be assessed it is essential to determine the degree to which the contamination of human diet depends on the rate of fall-out as opposed to the cumulative total in soil. In an earlier publication it was concluded that the contamination of milk, especially, depended largely on the rate of fall-out[1]. The very tentative nature of the assessment was, however, emphasized. A more precise assessment is now made possible by a more detailed study of the content of strontium-90 in human diet in the United Kingdom which was made in 1958[2] and by additional results of field experiments[3,4].

More than 40 per cent of the milk produced in the United Kingdom is now sampled every two weeks and bulked according to a statistical plan before analysis. During 1958 it was found that the ratio of strontium-89 to strontium-90 in milk during the summer months was more than five times that in the cumulative deposit and at least half of that in the current deposition in rainfall. Because of the shorter half-life of strontium-89 this ratio decreases by a factor of two every seven weeks; thus the effective delay period between the deposition of strontium-90 and its appearance in milk was 1–2 months. Evidence of the importance of recently deposited strontium-90 in determining the contamination of other foods was also obtained.

Although such observations support the conclusion that the contamination of diet is at present largely determined by the quantity of fall-out deposited in the recent past, they do not enable the magnitude of the contribution from the cumulative total in the soil to be assessed. The ratio of strontium-89 to strontium-90 observed in milk is equally compatible with the contrary views that all the strontium-90 in milk was deposited some two months previously, or that it was equally divided between very recent and very old material; other evidence, however, makes the latter conclusion appear unlikely. If the situation is to be adequately interpreted, it is necessary to solve the equation:

$$C = xDp + yDt \qquad (1)$$

where C is the quantity of strontium-90 in the material under investigation, Dp and Dt are respectively the deposition during the life of the plant from which the strontium-90 was derived and the cumulative deposition in the soil, and x and y are proportionality factors.

For reasons previously discussed[1], it has been concluded that the most satisfactory method now available for solving this equation is to compare the observed levels of strontium-90 from world-wide fall-out in food products with experimental measurements of absorption from the soil. Since 65 per cent of the strontium-90 in diet in the United Kingdom comes from milk and milk products[2] the solution of the equation for this material is of primary importance.

Absorption of Strontium-90 from the Soil

A series of field experiments[3,4] has been in progress for three years, to provide information on the extent to which strontium-90 will be absorbed by crops grown under normal agricultural conditions on representative soil types in the southern part of Great Britain; the results are therefore considered to be applicable to England and Wales. Strontium-89 has been applied as a spray to the surface of the soil which is then cultivated to a depth of 4, 7 or 11 in. before crops are sown. Since the application of carrier-free strontium-89 as a fine spray can be regarded as simulating the deposition of radioactive strontium in rain, these experiments should form a reasonably satisfactory basis for assessing the extent to which plants will absorb strontium-90 which is deposited in world-wide fall-out. When ryegrass, or a ryegrass-clover pasture mixture, is grown under these experimental conditions, it is found that absorption is considerably reduced when the soil has been cultivated to the greatest depth. Thus the relationship between the quantity of strontium-90 in soil and the content of plants will change slowly with time as the strontium-90 penetrates more deeply into the soil. Furthermore, it has been observed in some experiments that irrespective of the depth of cultivation the ratio of strontium-90 to calcium in plants may decrease with the passage of time. Two calculations of the relationship between the quantity of strontium-90 in soil and the content of pasture plants have therefore been attempted:

Calculation A: The immediate situation. To estimate the situation likely to occur soon after strontium-90 has been incorporated in the soil, the results have been used for the entire season's crop when land has been cultivated to 4 in. after contamination. The distribution of strontium-90 down the soil profile caused by this method of cultivation is comparable to that now observed from world-wide fall-out in undisturbed soil.

Calculation B: The long-term situation. Deeper penetration of strontium-90 into the soil must be considered. This calculation is therefore based on the average results obtained at the end of the growing season when grass has been grown on land cultivated to 4, 7 and 11 in. after contamination.

Since the extent to which strontium-90 is absorbed from the soil is influenced by the availability of calcium in the soil, the results of the different experiments have been weighted according to the fraction

Table 1. Estimate of Mean Ratio of Strontium-90 to Calcium expected in Pasture Grasses under Field Conditions in England and Wales from Soil containing 1 mc./km.²

'Available' soil calcium (m.equiv./100 gm.)		> 15	10–15	5–10	< 5	Country-wide mean
Fraction of total area		0·53	0·19	0·24	0·044	
Experimental results : Mean Sr-90/Ca ratio ($\mu\mu$c./gm.) in ryegrass	Calculation *A*	0·70	1·26	2·72	8·01	
	Calculation *B*	0·49	0·67	1·90	3·75	
Contribution to country-wide mean Sr-90/Ca ratio ($\mu\mu$c./gm.)	Calculation *A*	0·37	0·24	0·65	0·35	1·61
	Calculation *B*	0·26	0·13	0·46	0·17	1·01

Calculation *A*, the situation shortly after contamination ; Calculation *B*, the long-term situation

of the total agricultural land in England and Wales which is of similar calcium status[5]. The country-wide mean values for the ratio of strontium-90 to calcium expected in pasture grass assuming that the soil contains one millicurie of strontium-90 per km.² have been derived (Table 1). The content of plants has been expressed as the ratio of strontium-90 to calcium and not as a concentration or as the percentage absorbed ; as these latter quantities will vary, depending on yield and on the environmental conditions during the growing season, they are not satisfactory bases for the calculation of the resulting levels in milk.

Fraction of the Strontium-90 in Milk which is attributable to Absorption from the Soil

Since the field experiments were carried out in the southern part of Great Britain, attention is here confined to assessing the probable extent to which absorption from soil is responsible for the contamination of milk in England and Wales. It has been shown that the quantity of strontium-90 deposited in different parts of the country varies closely with rainfall and measurements of the content of rainwater have been made[6,7]. The mean cumulative deposition of strontium-90 per km.² of land throughout the country can therefore be derived as 11·5 mc. in the middle of 1958. The analysis of soil samples from representative undisturbed pastures in different parts of the United Kingdom shows that on average approximately 65 per cent of the estimated cumulative deposition remained in the upper 4 in. of soil[2] and it is known that little strontium-90 had penetrated below this level. It therefore appears reasonable to assume that some 65 per cent of the deposition as estimated by measurements of rainfall was accessible to plants in the upper soil layers ; the mean level of strontium-90 per km.² of soil in pastures in England and Wales in July 1958 was therefore 7·5 mc.

It is thus possible from the data in Table 1 to estimate the mean ratio of strontium-90 to calcium expected in pasture plants as a result of absorption from the soil. In calculating the resultant levels in milk, account must be taken of the discrimination factor of 0·10–0·14 against strontium relative to calcium in their passage from the diet of cattle to milk[8], and of the fact that an appreciable fraction of the total diet of cattle is relatively uncontaminated (for example, concentrates) ; this latter fraction has been assumed to be 20 per cent. The ratio of strontium-90 to calcium expected in milk as the result of strontium-90 entering plants from soil has been calculated on this basis and compared with the levels observed in the country-wide survey of milk during 1958 (Table 2). In the calculation it has been assumed that the discrimination factor against strontium relative to calcium is 0·14 ; since the observed range of values is 0·10–0·14 some over-estimate of the contribution of the soil may have been made.

The calculation suggests that at the present time (calculation *A*) about 20 per cent of the strontium-90 in milk is due to absorption from the soil ; furthermore it appears that, if the present cumulative deposit were more thoroughly incorporated in the soil (calculation *B*), it would lead to a level in milk of approximately 12 per cent of that now due to world-wide fall-out. Since the quantity of available calcium in soil varies between different parts of the country, variations in the magnitude of the contribution from soil are to be expected. Separate calculations have therefore been made for the eight zones into which England and Wales have been divided for the study of radioactivity in milk[2]. It appears that the present contribution of absorption from the soil may range from 10 to 33 per cent of the observed levels in milk.

In this assessment no allowance has been made for the effect of future applications of lime to agricultural land. When land is of low calcium status the addition of lime significantly reduces the uptake of strontium-90. Some estimate of this effect can be made from the results of field experiments[3,4], and it has been calculated that future additions of lime at normal agricultural rates to land of low calcium status would reduce the average absorption of strontium-90 from soil throughout the country by approximately 10 per cent.

The proportionality factor x in equation 1 has been estimated (Table 3) by dividing the difference between the observed and calculated values for milk (Table 2) by the rate of fall-out during the year[7] ; y has been estimated by multiplying the country-wide mean levels in herbage (Table 1) by the discriminatory factor in the transfer of strontium and calcium to milk.

Conclusions

The results of the present calculation for the long-term situation may be compared with the earlier assessment based on 1957 data. The two estimates

Table 2. Comparison of the Mean Level of Strontium-90 in Milk produced in England and Wales during 1958 with that expected if Strontium-90 was absorbed only from the Cumulative Deposit in Soil

	Ratio Sr-90/Ca $\mu\mu$c./gm.	Percentage of observed value
Observed value from world-wide fall-out, 1958	6·84	
Calculated levels for absorption from soil		
Calculation *A*, immediate situation	1·35	20
Calculation *B*, long-term situation	0·84	12

Table 3. Proportionality Factors for the Estimation of Levels of Strontium-90 in Milk by the Equation $C = xDp + yDt$

Proportionality factor	Calculation *A*, immediate situation	Calculation *B*, long-term situation
x, Dependent on rate of deposition ($\mu\mu$c. Sr-90/gm. Ca per mc./km.² deposited per annum)	1·0	1·1
y, Dependent on cumulative deposition ($\mu\mu$c. Sr-90/gm. Ca per mc./km.² cumulative total in the soil)	0·23	0·14

C, Content of milk: $\mu\mu$c. Sr-90/gm. Ca; Dp, rate of deposition: mc./km.²/annum; Dt, cumulative total in the soil: mc./km.²; x and y, proportionality factors.

are not entirely independent since the results for some half the field experiments used in the present calculation provided the basis for assessing absorption from the soil in the earlier study. Confidence in this approach is, however, encouraged by the fact that the values for the proportionality factor y are little different in the two calculations (0·14 and 0·16). The present estimate for proportionality factor x is less than two-thirds of that previously estimated. This is considered to be due partly to removal of strontium-90 from the soil not being taken into account in the earlier calculation and partly to the fact, to which attention has been directed elsewhere[2], that during 1958 the contamination of milk and other agricultural produce did not rise as greatly as the rate of fall-out. It appears probable that this was because the higher rainfall in that year caused a larger fraction of the deposition to be removed from the plants in wash-off.

Although the present calculations are based on the fullest data now available, their imperfections are clearly apparent. The extrapolation to the country-wide situation from a limited number of field experiments is an inescapably inaccurate procedure. Moreover, alternative methods for utilizing the present data are possible though on balance they appear less reasonable. However, two independent lines of evidence, namely the ratios of strontium-89 to strontium-90 in milk and the present calculation, both indicate the importance of recent deposition as opposed to the cumulative total in determining the contamination of milk. While the calculations here presented have been made with respect to the situation in England and Wales only, the general similarity of the ratios of strontium-89 to strontium-90 in milk throughout the United Kingdom[3] suggests that they provide a reasonable guide as to the situation in the whole country.

It has previously been emphasized that local agricultural conditions and climate may cause wide variations in the direct contamination of vegetation[1]; thus the contribution of the cumulative total to the mean level in diet is likely to vary both between different regions and between seasons in any one locality. The present assessment is advanced solely as the best approximation now available for a single country. It is, however, of interest that comparable evidence of the importance of the rate of fall-out in determining the level in diet is available from elsewhere. The ratios of strontium-89 to strontium-90 in milk produced in the United States and in Canada during 1958 have been shown to be similar to those in the United Kingdom[9]. This does not imply that the situations are identical, since the average ratio of strontium-89 to strontium-90 in rain during the same period appears to have been somewhat higher in the United States than in the United Kingdom. However, it is evident that the effective delay period between the deposition of strontium-90 and its appearance in milk was still relatively short; suggestions[10] that the quantity of strontium-90 in milk in North America is determined by the cumulative total in the soil are not compatible with these observations. Finally, it may be noted that recent evidence from a number of sources suggests that the contamination of wheat and rice[2,11,12] and of green vegetables[2] with strontium-90 also depends largely on the extent of the recent deposition.

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PROGRESS OF CANCER RESEARCH

THE thirty-sixth annual report of the British Empire Cancer Campaign* is a massive volume of more than 600 pages dealing with the component reports from the institutes and individual investigators supported by the funds provided by the Campaign—£637,000 was spent on research in 1958. To attempt to summarize the results or even the trends of all the investigations in progress is no easy task; some idea of the current themes can be gained by simply cataloguing the different branches of work which are being pursued at the various institutions (see last year's review in *Nature*, **182**, 1547; 1958).

The researches described in the Report can be grouped into three categories: (*a*) clinico-pathology of cancer; (*b*) radiation-therapy and physics and radiobiology; (*c*) carcinogenesis and carcinogens, chemotherapy and the cytology, genetics, biochemistry and demography of cancer.

Only a few of the hundreds of investigations can be referred to in the present review.

The lung cancer–cigarette smoking problem, after the initial impetus given to its solution by the admirable papers of Doll and Hill, has dwindled into

* British Empire Cancer Campaign. Thirty-sixth Annual Report covering the year 1958. Part 1: The Chairman's Statement and the Accounts. Pp. ii+43. Part 2: The Scientific Report of the Researches undertaken by the Central Organization and Its Autonomous Councils in the United Kingdom, and by some of Its Affiliated Organizations Overseas. Pp. lviii+636. (London: British Empire Cancer Campaign, 1959.)

attempts to reproduce the postulated carcinogenic effect of cigarette smoking, using mouse and rat tissue, and into the detection or identification of carcinogens in smoke condensates.

Blacklock, continuing the studies initiated by the late Sir Ernest Kennaway, has been inoculating fractions of tobacco smoke condensate into the lungs of rats; these substances were phenols, wax, neutral fractions, carboxylic acids, nornicotyrine, nicotyrine, metanicotine, myosmine, nornicotine, neutral fraction minus hydrocarbons, hydrocarbons and bases minus nicotine. The histological effect induced by tobacco tar in the lungs of rats and guinea pigs has been to induce squamous metaplasia of the bronchial mucosa, sometimes with papillomatous changes.

Peacock, at Glasgow, has studied the incidence of lung adenocarcinoma in series of mice housed in a soot-free room and also in a room exposed to the unfiltered air of central Glasgow; he concluded that, "there is no evidence to support the idea that atmospheric pollution is a major factor in the ætiology of adenocarcinoma of the lung in C_3Hf mice. Even in animals with no detectable soot in their lungs, tumours occurred at least as frequently as in those whose lungs had obvious deposits of soot". At the same centre polycyclic hydrocarbons present in diesel and petrol exhaust fumes have been identified and assayed: these were, 3 : 4-benzopyrene, 3 : 4-benzofluorene, tetracene (naphthacene), pentaphene, 1 : 2 : 3 : 4-dibenzopyrene, 11 : 12 benzofluoranthene, 1 : 2 : 9 : 10-dibenzotetracene, 1 : 2 : 4 : 5-dibenzopyrene and 1 : 12 : 2 : 3-dibenzoperylene.

Lasnitski, in Cambridge, has been studying the effect of cigarette smoke condensates on organ cultures of (i) human fœtal lung and bronchial tissues and (ii) the bronchi of newly born pigs.

At Leeds, Green and his collaborators are confirming Wynder's results using cigarette smoke condensate applied to mouse skin; so far as the experiments go, they show that cigarette smoke contains a weak carcinogen.

The effect has been tried of adsorbing a powerful carcinogen (9 : 10-dimethylbenzanthracene) on carbon particles and exposing mice to an atmosphere containing such an artificial soot; from the development of superficial papillomas it was concluded that the skin apparently elutes the carcinogen from the carbon, but that this elution does not take place in the subcutaneous tissues, nor do the mice breathing air loaded with the carbon-adsorbed carcinogen develop lung cancer. This result could suggest that the carbon, or soot, might conceivably have protective properties by the adsorption of carcinogens.

Among the latest additions to the catalogue of carcinogens is ordinary 'Cellophane', first discovered by Oppenheimer to induce sarcoma in rats after subcutaneous implantation. Horning and Alexander, and also Price, Geffree and Taylor, find that the tumour-producing potency depends very largely on the area of 'Cellophane' inserted under the skin; pieces of 9 cm.^{2} gave 46 per cent sarcomas, 2·25 cm.^{2} gave 19 per cent sarcomas and 0·56 cm.^{2} gave 0 per cent; filter paper (2·25 cm.^{2}) or micro cover glasses gave 0 per cent tumours. In Horning and Alexander's experiments with Visking sausage tubing, 2 cm. × 2 cm. gave 60 per cent sarcomas in rats; 1 cm. × 1 cm., 20 per cent and 0·5 cm. × 0·5 cm., 0 per cent. Oppenheimer, in 1955, had reported that if the 'Cellophane' is first perforated before implantation the incidence of tumours is very considerably reduced. Developments in this field of 'physical carcinogenesis' are awaited with much interest.

The more recent chemotherapeutic agents used for cancer treatment are *nor*-HN2(di-2-chloroethylamine), amino-chlorambucil, melphalan, busulphan (myleran) and glucosaccharo : $1 \rightarrow 4$: lactone. Unfortunately, these drugs have as yet had only a very limited degree of success, partly perhaps because the therapist usually tries them on advanced cases, and as a result of his caution he is likely to weight the chances against the treatment.

But if the cure of cancer by drugs is still in its infancy, the alternative approach to the disease, namely, prevention, is at least receiving more support. Doll and Hill came to the conclusion that in Britain four-fifths of lung cancers in men are inescapably connected with cigarette smoking: the Report quotes a parallel statement from Oettlé and co-workers who are engaged in demographic studies of cancer in South Africa. These workers write: "The figures provide further evidence of the influence of manner of life on susceptibility to particular cancers and would justify the conclusion that about two-thirds of white (that is, peoples) cancer deaths are inherently preventable by modification of manner of life and environment. The nature of such modification is for the most part not yet defined".

Oettlé *et al.* base their conclusions on the following data: the incidence of cancer in a population of half a million Bantu Africans was studied for a 3-year period and compared with U.S. White, U.S. Negro and Danish populations, age and sex being taken into account. It appears that urbanized Bantus have a total cancer-rate half that of U.S. Whites and Negroes, that the Bantu have a much higher œsophageal and liver cancer-rate (males) and a higher cervix-rate. Rural Bantu have a lower œsophageal and lung cancer-rate, but a higher sinus and foot melanoma-rate compared with urban Bantu.

The Cape Coloured population, which is of mixed White, Hottentot and Malay origin, shows a lower œsophageal and a higher lung and stomach cancer-rate than the Bantu; the manner of life of the Cape Coloured peoples resembles much more closely "that of Western races and their tumour incidence tends to resemble that of the American negro more than the South African Bantu".

Burrell, at the Cape Bantu Cancer Registry, reports that in the south-eastern regions of the province the natives have a very high and rising incidence of cancer of the œsophagus and that it appears to be more frequent among the well-to-do; he states, "Thirty-two instances of œsophageal cancer in man and wife have been recorded as well as other combinations of members of the same kraal household. A strong case is developing for environmental carcinogenesis". In the Bantu of the Mozambique region, Prates and colleagues report that liver cancer is exceedingly common whereas carcinoma of the œsophagus is unknown; skin cancer is very common and the general distribution of sites of cancer is very different from that in the Bantu of Johannesburg and the Transkei.

Another approach to the problem of cancer in Africa is the Kampala Cancer Registry, set up by the British Empire Cancer Campaign at Makerere College to study the incidence of cancer in Uganda. It has now been in operation for several years and is beginning to show practical results outside statistics. A method for the treatment of Kaposi's sarcoma by intra-arterial injection of nitrogen mustard has proved to be a

major advance in the treatment of this lesion, which is common in the area. One of the difficulties of the Registry has been the lack of adequate demographic data as a background to its own figures, and its committee appears to have stimulated a pilot survey of three small areas already completed, and of a survey of one whole country proposed as part of the current census of Uganda, which will result in knowledge of the age structure of the population in detail unusual in a tropical country. A similar registry has begun work in Jamaica, but has so far not reached the stage of reporting results. The value of surveys of this type may well be immense. As death-rates from malnutrition and infections decrease, tumours become more important in tropical areas, and it is well to be pre-warned as to what kinds of tumour are likely to become common: they present a very different pattern from those of Great Britain.

The most interesting development in the virus field in cancer in the past few years has been the investigation by Gross, who found that cell-free filtrates of tissues of the strain of mouse (*AKR*) liable to leukæmia, when injected into day-old C_3H mice, induced leukæmia and salivary gland cancer at a later stage. Salaman has now found that cell-free filtrates ('Gradocol') of leukæmic tissue from spontaneous leukæmia in *AKR* mice injected into 17 hr.-old C_3H mice induced 40 tumours in 15 out of 23 surviving mice; 22 were tumours of the salivary gland. The remainder were subcutaneous tumours of the thoracic and abdominal wall and were epithelial in type and closely imitated mammary cancer; a few were sarcomas.

Co-carcinogenesis has been the subject of experiments by Roe; he finds that the bladder as well as the skin of the mouse can be used as tissue for tests. Pellets of paraffin wax, alone or containing croton oil, were implanted in the bladder by the Bonser technique, and urethane was given by stomach tube in some of the experiments. When the bladder wall was examined (*post mortem*) after the tests had been carried on for 40 weeks, carcinomata, some of which were invasive, were found in a proportion of the mice. The yield of tumours, while appreciable, was insufficient for a precise assay; but it was obvious that both urethane and croton oil, alone or in co-carcinogenic combination, can induce papillomas and carcinoma of the mouse bladder epithelium.

As in previous Reports, a great deal of attention is being given at a number of centres to the study of deoxyribonucleic acid in normal and malignant tissue; one of the most interesting advances is described in the experimental work of Harris and his colleagues at Oxford. Along with other workers he has criticized the assumption made in the important researches of Schoenheimer that the components of all the tissues are in dynamic equilibrium; he points out that, "experiments on animals do not distinguish between intracellular turnover and turnover due to simultaneous multiplication and destruction of cells or to secretion of products by cells". Using the macrophage as an example of a non-multiplying cell and (8-^{14}C) adenine as tracer, he has shown that deoxyribonucleic acid is not synthesized in the cell, and no deoxyribonucleic acid adenine or thymidine was involved in turnover; but the ribonucleic acid was found to have a half-life of 2 hr. when it incorporated exogenous labelled adenine. Using tritium-labelled precursors, no transfer of ribonucleic acid from nucleus to cytoplasm could be detected and the half-life of nuclear ribonucleic acid was found to be 2 hr. both in macrophages and in connective tissue cells.

Table 1. Percentage Conversion of Added ^{14}C-labelled Glucose and Sugar Alcohols to Carbon-14 Dioxide

500 mgm. slices or cells incubated in Krebs–Ringer bicarbonate solution containing 20 mgm. labelled substrate (0·2 μc. ^{14}C) for 3 hr. at 37° C. Results are given ± S.E.M.

Tissue	D-Glucose	D-Sorbitol	D-Arabitol	D-Xylitol
Spleen	5·0±0·6	1·1±0·2	0·17±0·03	1·3
Thymus	3·5	—	0·1	—
Lung	2·6±0·3	0·70±0·03	< 0·05	—
Brain	9·0	0·50	0·11	—
Muscle	4·0	0·30±0·01	< 0·05	—
Lactating mammary gland	20·1	0·65	< 0·05	—
Kidney cortex	12·4±1·9	6·2±1·8	1·3±0·1	16·1±2·4
Liver	4·6±0·4	3·9±0·5	0·87±0·17	4·3±0·4
Fœtal liver	2·6±0·4	2·0±0·1	0·25±0·03	—
DAB hepatoma	4·1±0·7	1·4±0·5	0·15±0·03	2·1±0·1
β-Propiolactone tumour	7·0	0·62	0·12	—
Ascites tumour cells	4·7±0·4	0·87±0·08	0·10	—

It is now more than thirty years since the carbohydrate metabolism of tumours was used by Warburg as a basis for his hypothesis of the characteristics of malignancy; this theme is still a subject for experimentation and controversy. Glock and McLean have adapted the radiocarbon-labelling technique to the study of the glucose metabolism of normal tissues and tumours. Their results are shown in Table 1, and suggest that when rat liver becomes converted to hepatoma by dimethylaminoazobenzene (DAB), some of the characteristics of the carbohydrate metabolism are lost; but in this respect the resulting malignant tissue can scarcely be distinguished from some other normal tissues. Results for the metabolic capacities of the normal tissues (if obtainable), which were the origins of the β-propiolactone-induced tumour and the ascites tumour, would constitute the essential material for adequate comparison.

Physicists become more and more concerned with less in the field of radiation, and a very large number of the reports from physicists working in this field of cancer research are concerned with identifying and measuring amounts of radiation that are considerably less than those met with in the more usual radiological or radiotherapeutic fields. Very keen interest is being taken in environmental radioactivity, and reports of the measurement of the activity from building materials have been supplemented by those dealing with ingested naturally occurring radioactivity. It is reported that Brazil nuts contain more than 20,000 times the activity found in other common foodstuffs. New and more sensitive methods of detection of β- and γ-radiations have been produced, so that it is now possible to detect as little as 20 μμc. cæsium-137 in a single human milk sample. These advances are of great importance in the study of the radiation-levels received by the population in various areas depending on their dietary habits, on the geological nature of the area in which they live and on the type of materials used in the buildings in which they live and work.

The results of experiments carried out to discover the effects of prolonged irradiation at low levels of dosage on mice indicate a premature ageing under such experimental conditions, and this finding confirms the need for such research into the nature of the irreducible natural radioactive background which is present before the addition of increased man-made

radioactive contamination into the daily lives of a large proportion of the population.

The radiation dosimetry in bone from bone-seeking radioactive isotopes is being studied in several centres, and practical confirmation is being obtained of the theoretical calculations which have been reported previously. The correlation of the dose received by the sternal marrow during irradiation treatment with the hæmatological findings in such sternal marrow samples is also being studied. Experiments are also reported which are designed to provide further confirmation of Dr. R. H. Mole's observations that the important factor in the production of radiation leukæmia is the dosage-rate rather than the total dose received.

Confirmation of the advantages of radiotherapy with megavoltage radiation is reported from centres all over the world, and the installation and calibration of such equipment necessitate a considerable amount of time being spent on the basic physical measurements which are necessary before the equipment can be put into routine use.

Instruments for the continuous integration and recording of solar erythemal ultra-violet radiation have been designed in Queensland, Australia. This equipment will be used in conjunction with a survey which is now being made of various aspects of the occurrence of skin cancer in an attempt to assess the chief factors causing skin cancer.

Work is also continuing in several centres on the response of tumours to irradiation in atmospheres of various concentrations of oxygen. The radiosensitivity of normal and of malignant cells alike has been found to be under the control of the concentration of dissolved oxygen in the immediate environment of the cell. The administration of oxygen under pressure aims at raising tissue oxygen tension and hence tumour sensitivity by supercharging the plasma with dissolved oxygen.

An interesting application of the Sandberg–Moore technique for detecting tumour cells in venous blood is reported from the Middlesex Hospital, London. In addition to samples of peripheral venous blood and of blood from veins draining the tumour, samples of hepatic vein blood were taken by trans-cardiac catheterization in order to assess the ability of the liver to clear tumour cells from the blood passing through it. The nine cases of gastro-intestinal cancer in which samples were taken from both portal vein and hepatic vein are of particular interest. Tumour cells (certain or suspicious) were seen in eight cases in the portal vein and in only two in the hepatic. Though the numbers are small, they strongly suggest a very substantial filtration effect by the liver. Curiously enough, even in gastro-intestinal tumour, the peripheral blood appears to be more often positive than the hepatic vein, and though the numbers are again too small to be reliable, this would if confirmed suggest in the first place a substantial by-pass of the liver (perhaps by tumour cells passing up the thoracic duct) and in the second place that the lung is a very much poorer filter than the liver.

A group at Mount Vernon Hospital presents interesting findings on the deoxyribonucleic acid content of the nuclei of tumours, using micro-spectrophotometry of Feulgen-stained preparations. Of 100 carcinomata of the cervix uteri, 40 had modal values at or near the normal diploid value, and 32 near the tetraploid value. No clear relation between ploidy and degree of differentiation was noted, though adenocarcinomata had high figures and both of two tumours of mixed histology had very variable nuclear deoxyribonucleic acid with no defined mode. Limited observations on recurrent tumours suggest that high-count tumours are more resistant to radiation and that recurrences have a higher count than the original tumour. In tumours from women, the presence of the sex chromatin (the small body which represents the heterochromatin of the *XX* chromosome pair in man and some other animals) can be roughly correlated with the degree of ploidy, being usually present in diploid and often double in tetraploid tumours.

OBITUARY

Mr. E. W. B. Gill, O.B.E.

Ernest Walter Brudenell Gill died on December 20 at Watlington, Oxon, after a long illness, at the age of seventy-six. Born in London, the son of a canon, educated at Bristol Grammar School and Christ Church, Oxford, where he was an Open College Exhibitioner in mathematics and later a Scholar, he took a first-class in physics in 1905, a B.Sc. degree in 1909, became University demonstrator in the Electrical Laboratory under Townsend, and a Fellow (1910) and later the domestic bursar of Merton College, Oxford.

During the First World War he served in the Royal Garrison Artillery and the Royal Engineers Wireless Intelligence, which took him to the Middle East. Afterwards he wrote a witty little book, "War, Wireless and Wangles". During the Second World War he again was in Intelligence and operational research. He was a co-inventor of 'window', metal foils dropped from planes so that our aircraft could not be located by enemy radar; he also worked on devices for guiding tanks. After the War he and an American representative interviewed the German scientists. He was surprised to find how little they had contributed to the war effort, some pretending that this was to save the world from destruction. A humorous account of his experiences, intended as a book, remains in the manuscript stage.

Gill's numerous scientific contributions cover a wide range of physical problems. With Pidduck he measured the ionization coefficient in helium. He discovered the effect of the electric field on the saturation current of tungsten emitters in valves and developed an ingenious method of determining the high-frequency conductivity and dielectric constant of the soil by measuring the ratio of the vertical and horizontal components of electric field and the phase angle between them. He investigated extensively electrodeless discharges in high-frequency electric fields, and observed that at very low gas pressure the starting potential becomes very small when a magnetic field applied perpendicular to it is of such value that electrons move through their orbit in a time equal to their periodic time (1937), an effect

recently spectacularly demonstrated by Cutolo in Naples, who started a discharge a long distance from the transmitter of an electromagnetic beam. With me he found that in zero magnetic field the initial multiplication of charges at low pressure is due to secondary electrons released from the walls of the glass vessel. He contributed greatly to our knowledge of static electrification, showed that frictional electricity is caused by contact potentials and found that by freezing-in charges in ice surrounded by water, potential differences of several 100 volts appear across the ice. Later he turned his fertile mind to the elucidation of the mechanism of the rise of sap in trees.

Gill was an outstanding Englishman; a gay character, modest, with an inborn sense of humour and a fund of excellent stories. He had the original mind of a young man coupled with the neat approach of a professional soldier. Debunking ("rebuking sin") was his hobby. Because of his independent judgment, his unfailing logic and his devastating remarks (often noted by the Press), he stepped quite often on people's toes. He never received the recognition he deserved either as a scientist or for his work in the administration of the University or as an Alderman of the City. He was a trusted friend and a devoted husband, who is survived by his wife, three sons and one daughter.

A. von Engel

NEWS and VIEWS

National Agricultural Advisory Service : W. Morley Davies

Mr. W. Morley Davies has retired from his post of senior advisory officer in the National Agricultural Advisory Service. He attended the University of Birmingham, taking a degree in chemistry and botany. During the First World War he was engaged on the manufacture of explosives in British Dyestuffs, Ltd., Huddersfield. Afterwards, he went to Gonville and Caius College, Cambridge, as a research student and worked in the School of Agriculture under Prof. T. B. Wood and Arthur Amos, taking a research B.A. in 1922. At the same time, he also obtained the agricultural diploma. In September 1922 he was appointed head of the Department of Chemistry at the Harper Adams Agricultural College, and later, in 1924, was also appointed as advisory agricultural chemist to the West Midland Province. During that period, mainly in collaboration with the late Prof. G. W. Robinson, Prof. T. Wallace, Sir William Ogg and others, many of the methods now in use in the Soil Survey were developed. In 1946 he was appointed the first director of the East Midland Province in the newly constituted National Agricultural Advisory Service. He was appointed senior education and advisory officer in charge of all the science sections of the Service and stationed at headquarters in London in 1949. As chairman of the Land Restoration Committee and also of the Technical Advisory Conference on the Restoration of Opencast Coal Land, he piloted much work on the technical problems of restoring land to agriculture after it had been subjected to mineral working. He was also a member of the Ironstone Advisory Committee appointed by the Ministry of Housing and Local Government. During the period at headquarters he was secretary of the Agricultural Improvement Council (England and Wales) and assessor for the Ministry of Agriculture on several of the committees of the Agricultural Research Council as well as on the Geological Survey Board.

Dr. N. H. Pizer

Dr. N. H. Pizer, who will succeed Mr. Morley Davies, has been regional soil chemist in the Eastern Region of Great Britain since 1946 and has been interested in soil conditions affecting root development and nutrition of crops, particularly those related to soil conformation and structure. Physical defects such as compacted or structureless layers have been found to occur in many soils and to impede drainage, root development and root activity. Some are due to treatment of the soil and others are natural, and their effects on crops vary with season. Methods of dealing with these defects have been worked out with the farmers concerned. Some defects have required drainage operations or cultivations to a suitable depth when the soil was dry, and others a combination of the two. Dr. Pizer has also worked on the amount and distribution of organic matter in soils under different systems of husbandry and the effects which very low levels of organic matter may have on the physical condition of soils and their ease of working. Another of his interests has been the occurrence of trace-element deficiencies in crops and the development of methods of recognition and treatment. As a result of this work, copper deficiency was found to occur in cereals growing on many fen peat soils and on very light sandy soils previously reclaimed from heath. Yields of cereals were increased from a few cwt. an acre to more than 30 cwt. by the application of ¼–1 lb. of copper per acre as a foliar spray.

After the sea floods of 1953 which affected most of the coast-line of eastern England and extended inland up the river valleys, considerable work was done in the field and laboratory on methods of restoration of soils. Much variation was found between soils in the effects of salt on their physical condition, some being extensively damaged and others not at all, and these variations had an important influence on the treatment that was recommended to restore the land to its former condition. More recently, the problem of magnesium deficiency in fruit crops, glasshouse crops and arable crops on light land has been studied and practical methods of treatment developed.

Paul Instrument Fund : Awards

Awards of grants by the Paul Instrument Fund Committee have been made as follows: £500, in supplement of a previous grant, to Dr. B. V. Bowden, principal of the Manchester College of Science and Technology, for the development of narrow pass-band dye filters; £545, in supplement of a previous grant, to Dr. R. Feinberg, of the Department of Electrical Engineering, Manchester College of Science and Technology, for the construction of an instrument for measuring the intensity of soft X-radiation generated in experimental high-voltage valves; £3,000 to Prof.

D. Gabor, professor of applied electron physics in the Imperial College of Science and Technology, London, and Dr. D. Jones, of the Department of Electrical Engineering in the Imperial College, for the development of an electron interference microscope; £8,100 to Dr. H. H. Hopkins, reader in optics, Department of Physics, Imperial College of Science and Technology, London, for the development of methods of drawing and coating fine glass fibres, and their alignment in ordered bundles for the transmission of optical images; £3,000, in supplement of a previous grant, to Prof. R. O. Redman, professor of astrophysics in the University of Cambridge, for the construction and testing of a thin astronomical mirror of plate glass and of a new type of support system; £6,000, in supplement of a previous grant, to Dr. C. N. Smyth, of University College Hospital Medical School, London, and Mr. F. Y. Poynton, of Northampton College of Advanced Technology, London, for the construction of an ultrasonic microscope.

The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Physical Society, the Institute of Physics and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

Commonwealth Scholarship Commission in the United Kingdom

THE following have been appointed members of the Commonwealth Scholarship Commission in the United Kingdom by the Secretary of State for Commonwealth Relations, Lord Home, and the Secretary of State for the Colonies, Mr. Ian Macleod: The Earl of Scarbrough, the Lord Chamberlain (*chairman*); Dr. R. S. Aitken, vice-chancellor of the University of Birmingham; Sir David Anderson, recently director of the Royal Technical College, Glasgow; Sir Eric Ashby, master of Clare College, Cambridge; Sir Eric Coates, Finance Minister of the Governor General of India's Executive Council, 1946; Lord Geddes of Epsom, member of the London Transport Executive; Sir Hector Hetherington, principal and vice-chancellor of the University of Glasgow; Sir Francis Hill, chairman, General Purposes Committee of the Association of Municipal Corporations; Sir Cyril Hinshelwood, Dr. Lee's professor of chemistry in the University of Oxford, and president of the Royal Society; Sir Douglas Logan, principal of the University of London; Lady Ogilvie, principal of St. Anne's College, Oxford; Sir Julian Pode, managing director of the Steel Company of Wales, and chairman of the Managing Committee of the United Kingdom for the Athlone Fellowships scheme; Sir Paul Sinker, director general of the British Council, and First Civil Service Commissioner, 1951–54; Dr. R. W. West, principal of Battersea College of Technology. The Commission has been set up under the Commonwealth Scholarships Act recently passed by Parliament to administer the Commonwealth Scholarship and Fellowship Plan in Great Britain.

A Hundred Years of Optics

IN 1860 John Henry Dallmeyer took out a patent and issued his first Triple Achromatic lens, marking the foundation to the House of Dallmeyer. J. H. Dallmeyer was born in Westphalia in 1830 and went to Great Britain in 1851. He first set up a business designing and manufacturing lenses and optical instruments at 19 Bloomsbury Street, London. In 1861 he was elected a Fellow of the Royal Astronomical Society, and four years later invented the Dallmeyer Rectilinear lens. Improvements in optical technology brought about the series of *f*/3, *f*/4 and *f*/6 Patent Portrait lenses. The management of the business gradually devolved upon Thomas Rudolph Dallmeyer, who, like his father, possessed a wealth of knowledge in optics. In 1891 the first practically useful telephoto lens was designed and issued by him, only to be followed by the Adon telephoto lens in 1892. One of the earliest treatises on the use of telephoto lenses was to his credit, and "Telephotography" was published in 1899. T. R. Dallmeyer died in 1906, and it was only due to the fact that a private limited company had been formed in 1892 that the business remained established. Later developments included the pioneering of the first fixed-separation telephoto lens, the inventions of the Dallon Fixed-separation Telephoto lens and the Pentac anastigmat lens. It was the Pentac lens that created new standards in large-aperture anastigmal lenses, and which was used during the Second World War for aerial reconnaissance both by day and night. It is with much credit to its pioneers that the House of Dallmeyer celebrates its centenary.

Public Health Laboratory Service in Britain

THE Public Health Laboratory Service Bill, which received its second reading in the House of Lords on February 4, transfers the administration of the Public Health Laboratory Service in Britain from the Medical Research Council to a new Public Health Laboratory Service Board appointed by the Minister of Health and subject to his directions. The Board will consist of a chairman and nine other members, one of whom must be medically qualified; two members will be appointed after consultation with the Medical Research Council, two must have had experience as bacteriologists, two will be medical officers of health of local authorities, and two with administrative experience. Clauses in the Bill transfer the staff of the Public Health Laboratory Service from the Medical Research Council to the new Board, and property held for the Public Health Laboratory Service is transferred to the Minister of Health. In introducing the Bill, the Minister for Science, Lord Hailsham, explained that the Service had its origin in an emergency service created shortly before the Second World War at the request of the Committee of Imperial Defence, in order to provide a defence against possible enemy use of bacteriological warfare and against any major outbreaks of epidemic diseases arising from war-time conditions. In the event it was never used to deal with any such major emergency, but it proved a very valuable aid to the medical officers of health in their ordinary work, in view of the inadequacy of the arrangements for bacteriological work for public health purposes. Unlike the Hospital Laboratory Services, it was organized on a national and not on a regional or hospital basis, and consists at present of the Central Public Health Laboratory, eight regional and 51 area laboratories, with several special reference libraries; of the staff of about 1,068, 126 are medically qualified. £1,212,000 was provided for the cost of

the Service in the Civil Estimates for 1959–60 and another £50,000 for capital expenditure on buildings. Replying to Lord Taylor, who paid tribute to the immense amount of research carried out by the Service (estimated at about one-quarter of its work), Lord Hailsham readily gave the assurance requested that its research activities would not be curtailed.

British Overseas Information Services

In the House of Commons on February 1, the Chancellor of the Duchy of Lancaster, Dr. C. Hill, gave the estimated cost of the overseas information services during 1959–60 as £16,966,140, of which 30·3 per cent was attributable to the British Council, 39 per cent to the British Broadcasting Corporation's External Services, 18·3 per cent to the Foreign Office, 8·4 per cent to the Commonwealth Relations Office, and 3·7 per cent to the Colonial Office. There were 117 United Kingdom information posts overseas, of which nine were staffed by one United Kingdom-based officer only, and three by one locally-recruited officer only.

Research and Industry in Great Britain

In reply to a question in the House of Commons on February 4, the Minister of Education, Sir David Eccles, as representing the Minister for Science, said that the research needs of the printing machinery, textile machinery and shipbuilding industries were among those being considered for special study by the Department of Scientific and Industrial Research. Since the full co-operation of the firms concerned was essential, an inquiry on a confidential basis was proposed. The report would afterwards be discussed by the industry and steps taken to remedy any defects.

Courses on Nuclear Energy for Engineers and Technical Industrialists

Two international courses on nuclear energy, designed for senior engineers and technical industrialists, are being arranged this summer by the European Nuclear Energy Agency of the Organization for European Economic Co-operation. One course will be at the Centre d'Etudes Nucléaires at Saclay (France) and the other at the Atomic Energy Research Establishment, Harwell. Each will be open to some fifty participants from countries belonging to the Organization, and will cover the technical, economic and industrial aspects of nuclear energy. The course at Saclay (in French) will be held during June 27–July 8; the Harwell course (in English) during July 4–15. The programme in each case will comprise lectures, practical work and visits to installations, and will be presented throughout from the point of view of the engineer and the industrialist. Further information can be obtained from O.E.E.C. European Nuclear Energy Agency, 38 Boulevard Suchet, Paris 16.

Courses in Advanced Technology

The wide range of part-time courses dealing with advanced aspects of technology provided by technical institutions in the London area is described in a bulletin prepared by the London and Home Counties Regional Advisory Council for Technological Education. At Balham and Tooting College of Commerce experts from foreign embassies were invited to discuss the problems of British export policy. A course on energy approximations in structural design was arranged at Battersea College of Technology, while at the Borough Polytechnic bakery sales managereses were invited to learn how better to sell bakery products. Joinery estimating was available for joinery managers and shop foremen at the Brixton School of Building, and new developments in gas chromatography were available at the Brunel College of Technology. At the City of London College senior executives of tanker operating firms were invited to a series of lectures on the economics of tanker shipping operations. Further details of the bulletin can be obtained from the Secretary, Regional Advisory Council, Tavistock House South, Tavistock Square, London, W.C.1.

The Leopoldina German Academy of Sciences: Elections

The Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members, in the sections indicated: *Zoology*, Prof. G. P. Baerends (Haren (Gron.) Holland), Prof. Willi Hennig (Berlin), Prof. Werner Jacobs (Munich), Dr. Martin Lindauer (Munich), Prof. Hans J. Müller (Quedlinburg), Dr. Johann Schwartzkopff (Munich), Prof. Niko Tinbergen (Oxford); *Physical Chemistry*, Prof. Gustav Kortüm (Tübingen), Prof. Werner O. Köster (Stuttgart); *Internal Medicine*, Prof. Ferdinand Hoff (Frankfurt/M.), Prof. Hugo Wilhelm Knipping (Cologne), Prof. Ernst von Lauda (Vienna), Prof. Paul Martini (Bonn), Prof. Helmuth Reinwein (Kiel), Prof. Rudolf Schoen (Göttingen).

Clough Memorial (Geological) Research Fund

The Clough Memorial Research Fund of the Edinburgh Geological Society is available for geological research in Scotland and the North of England (Northumberland, Durham, Cumberland, Westmorland and Yorkshire). Grants for the period April 1, 1960–March 31, 1961, will be made shortly, and applications, stating the nature of the proposed research, should be sent before March 31 to the Secretary, Clough Memorial Research Fund Committee, Edinburgh Geological Society, Synod Hall, Castle Terrace, Edinburgh 1.

University News: London

Dr. H. O. Schild, reader in pharmacology at University College, has been appointed to the University chair of pharmacology tenable at that College, as from October 1, 1961; and Dr. W. F. Widdas, reader in physiology at King's College, has been appointed to the University chair of physiology tenable at Bedford College, as from October 1, 1960. The following appointments are also announced: Dr. H. Lehmann, senior lecturer in chemical pathology at St. Bartholomew's Hospital Medical College, to the University readership in chemical pathology tenable at that College; and the title of reader in chemical pathology in the University of London on Dr. G. R. Webster in respect of his post at Guy's Hospital Medical School.

Oxford

It is announced that the Forestry Commission is to make a grant not exceeding £4,000 for two years beginning April 1, 1960, for the continuation of an investigation into the costs of private forestry and economic information concerning private forestry estates in England and Wales, being carried out in the Department of Forestry under the direction of J. J. MacGregor.

The Night Sky in March

FULL moon occurs on Mar. 13d. 08h. 26m. U.T., and new moon on Mar. 27d. 07h. 38m. The following conjunctions with the Moon take place: Mar. 5d. 00h., Aldebaran 0·4° S.; Mar. 20d. 11h., Jupiter 5° S.; Mar. 21d. 14h., Saturn 4° S.; Mar. 24d. 04h., Mars 4° S.; Mar. 25d. 12h., Mercury 0·7° S.; Mar. 25d. 12h., Venus 2° S. In addition to these conjunctions with the Moon, Mercury is in conjunction with Venus on Mar. 25d. 09h., Mercury being 1·9° N. There will be a total eclipse of the Moon on March 13 and a partial eclipse of the Sun on March 27, both eclipses being invisible at Greenwich. Mercury can be seen during the first few days of the month as an evening star, but conditions rapidly become unfavourable, and after March 5 Mercury will be too close to the Sun for easy observation. There is an occultation of Mercury by the Moon on March 25, visible from Greenwich and lasting from 11h. 51·1m. to 13h. 03·6m. Venus and Mars are too close to the Sun for easy observation. Jupiter is a morning star, rising at 3h. 30m., 2h. 40m. and 1h. 45m. on March 1, 15 and 31, respectively. It may be seen in the south-east before sunrise; it is in Sagittarius. Its stellar magnitude is −1·7 and its distance from the Earth in the middle of the month is 490 million miles. Saturn is a morning star, rising at 4h. 30m., 3h. 40m. and 2h. 40m. on March 1, 15 and 31, respectively. It is visible in the south-east before sunrise, and, like Jupiter, is in Sagittarius. Its stellar magnitude is +0·8 and its distance from the Earth on March 15 is 960 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Mar. 4d. 20h. 40·6m., 6^1 Tau. (*D*); Mar. 4d. 20h. 44·2m., 75 Tau. (*D*); Mar. 4d. 21h. 40·3m., 264 B. Tau. (*D*); Mar. 5d. 00h. 00·2m., 111 Tau. (*D*); Mar. 11d. 3h. 18·0m., o Leo. (*D*); *D* refers to disappearance. The vernal equinox occurs on March 20d. 15h.

Announcements

A DECISION by the U.S.S.R. to join the International Federation of Computer Societies brings the number of nations which have ratified the statutes of this new international organization up to twelve. The Academy of Sciences of the U.S.S.R. will be represented in the Federation by its Computing Centre.

AN international symposium on Growth: Molecule, Cell and Organism will be held at Purdue University, Lafayette, Indiana, during June 16–18. Further information can be obtained from Prof. M. X. Zarrow, Department of Biological Sciences, Purdue University.

THE annual International Starch Conference is to be held at the West German Starch Research Station, Detmold, during April 20–22. Further information can be obtained from W. Kempf, Cereal Research Station, P.O. Box 23, Detmold, West Germany.

A CONFERENCE on the Computing Laboratory in the Technical College is to be held during May 27–28 at the Hatfield Technical College, which is sponsoring it. Further details can be obtained from W. A. J. Chapman, Department of Mathematics, Hatfield Technical College, Hertfordshire.

THE Chemical Society is organizing a symposium on Physical, Chemical and Biological Methods of Study in High Molecular Weight Carbohydrates, to be held in Edinburgh during July 12–14, 1960. Further information can be obtained from the General Secretary, Chemical Society, Burlington House, London, W.1.

THE fifth International Congress on Nutrition will be held in Washington, D.C., during September 1–7, under the auspices of the International Union of Nutritional Sciences. Dr. C. Glen King (The Nutrition Foundation, New York City) will preside over the Congress. Further information can be obtained from Dr. Milton O. Lee, 9650 Wisconsin Avenue, Washington 14, D.C.

THE general programme of the Madrid Sectional Meeting of the World Power Conference during June 5–9 has now been published. British subjects wishing to obtain further information should write to the Secretary, British National Committee, 201–2 Grand Buildings, Trafalgar Square, London, W.C.2, from whom copies of the programme can also be obtained.

A CONFERENCE on Solid State Microwave Amplifiers is being organized by the Electronics Group of the Institute of Physics, in conjunction with the Radio Spectroscopy Group, at the University of Nottingham during April 6–8. Further details can be obtained from the Secretary, Institute of Physics, 47 Belgrave Square, London, S.W.1.

THE International Medical Congress, Ltd., which has a strong affiliation with the National Foundation of New York, is holding its first International Conference on Congenital Malformations in London during July 18–22. Further information can be obtained from the Executive Secretary-Treasurer, First International Medical Conference on Congenital Malformation, 67 New Bond Street, London, W.1.

THE Pesticides Group of the Society of Chemical Industry is organizing a symposium on Fungicides in Agriculture and Horticulture in the spring of 1961. It would be appreciated if workers on these subjects who may wish to submit a contribution to this symposium would get in touch with the hon. secretary of the Pesticides Group, Dr. B. J. Heywood, c/o May and Baker, Ltd., Dagenham, Essex, giving a very brief indication of the scope they would wish to cover.

PROF. R. A. MCCANCE and the Department of Experimental Medicine, Cambridge, are arranging a course on the Clinical Aspects of Nutrition, to be given in the Department of Geology, Cambridge, during April 6–9. The course is open to university and hospital medical and scientific staffs and registered medical practitioners. Further information can be obtained from the Secretary, The Medical School, Tennis Court Road, Cambridge.

THE second European symposium on Chemical Reaction Engineering is to be held at the Koninklijk Instituut voor de Tropen, Amsterdam, during April 28–30. The symposium is being organized under the auspices of the European Federation for Chemical Engineering by the two Dutch member societies. Further information can be obtained from P. J. Hoftyzer, Central Laboratory, Staatsmijnen in Limburg, Geleen (L), Netherlands.

ERRATUM. We regret that in the communication entitled "Shape of the Precipitate in Immunoelectrophoresis", by Dr. Jan Hirschfeld, in *Nature* of January 16, p. 164, Fig. 4 has been printed upside down.

THE INTERNATIONAL COMMITTEE ON LABORATORY ANIMALS

By Dr. W. LANE-PETTER

Laboratory Animals Centre, Carshalton, Surrey

In December 1956, the International Committee on Laboratory Animals was formed, and a report of this event appeared two months later in *Nature* (179, 240; 1957), describing its background, composition and purpose. After three years, it is timely to review its work and achievements.

The Committee is to-day supported, by direct grant and by representation, by the Council for International Organizations of Medical Sciences: the International Unions of Biological and of Physiological Sciences; and by direct assistance other than cash grants by Unesco. It consists of: Prof. J. H. Maisin (Belgium), chairman of the Committee and representative of the Council for International Organizations of Medical Sciences; Prof. W. K. Hirschfeld (Holland), vice-chairman; Prof. G. Montalenti (Italy), representing the International Union of Biological Sciences; Prof. J. A. B. Gray (United Kingdom), representing the International Union of Physiological Sciences; three members co-opted under the by-laws for a two-year term from October 1958: namely, Dr. Dale W. Jenkins (United States), representing the International Committee on Laboratory Animals in the American Continent; Monsieur M. Sabourdy (France), treasurer; and Dr. W. Lane-Petter (United Kingdom), secretary-general; and an observer from Unesco. Meetings of this Committee have been held in December 1957 (Paris), October 1958 (Paris) and April 1959 (London). The next meeting will be held in April 1960 in Rome.

Already in December 1956, Unesco had initiated surveys on the production and utilization of laboratory animals in a number of countries, and further surveys have since been commissioned and carried out, on the advice of the Committee. Some have been published, in consolidated form, by the Committee as mimeographed documents: Vol. 1 including India, Italy, Japan, Switzerland and the United Kingdom: Vol. 2, Denmark, Finland, Iceland, Norway, Sweden, and Turkey (first supplement); Federal Republic of Germany and Austria (second supplement); and Australia (third supplement). A third volume will include Belgium, Netherlands, France, Israel, Poland, Czechoslovakia, and probably a partial survey of the United States.

From these surveys has come a clear picture of the actual production and utilization of laboratory animals in many different countries; of the varying special problems, and of the underlying basic needs, which are everywhere the same; and an indication of the way in which an international organization such as the Committee can make itself most useful.

As a further attempt to clarify the task that the Committee had undertaken, a symposium was held in Paris in October 1958; a report of this is still in preparation, but will appear very shortly. At the symposium three main questions were examined. First, what were the detailed requirements in the main biological disciplines using animals: namely, cancer research, radiobiology, microbiology, physiology, cell and tissue culture, endocrinology, and pharmacology and the pharmaceutical industry. Secondly, to what extent were these needs being met to-day in different countries. Thirdly, what were the future possibilities, with special reference to genetics, the control of latent infections, the introduction of new species into laboratory use, and the problems of large-scale production of familiar species.

Since September 1957, the Committee's *Bulletin* has appeared regularly every six months. It contains current news of the work of the Committee and also an excellent bibliographical service on laboratory animals.

Consultant members of the Committee have paid numerous visits to other countries, mainly in Europe, to advise on the creation or organization of national laboratory animal centres and to co-ordinate the efforts being made in this field. Indeed, this sort of personal contact has been of great value in stimulating interest, in encouraging the setting up of new national organizations and in linking the activities of all these in a common effort. In 1956, three national centres only existed, in the United Kingdom, the United States and France. To-day, centres of one kind or another have been formed in Australia, Belgium, the Federal German Republic, Holland, India, Italy, Japan, and more are likely to form in the near future. Almost all owe something, some even their very existence, to the stimulus provided by the Committee.

In 1959, Unesco placed a contract with Monsieur M. Sabourdy, treasurer of the Committee, for the preparation of a list of definitions of terms used in describing laboratory animals of high quality. Such definitions have been attempted in the past, notably among geneticists, but in the absence of a recognized code widely agreed, they tend to lose their exact meaning and in time to become almost valueless. A code of this kind is a necessary adjunct to drawing up specifications for animals; but it should be emphasized that attempts to draw up specifications should not wait on the completion of a glossary of terms, because the need to define many of the terms will become apparent only when specifications are attempted. There must be mutual reaction between the two endeavours, definition and specification.

Most of the surveys already referred to have directed attention to a universal need, namely, for animal technicians who are systematically trained and qualified for the work of breeding and maintaining the animals of high quality that research workers need in nearly every field. Great Britain has been the pioneer in this field, through the work of the Animal Technicians Association, now about ten years old, but training schemes for animal technicians are in operation in United States, France and Czechoslovakia. In the *Bulletin* of September 1959, detailed notes for training animal technicians were published, based largely on the British experience, but taking into account the different needs and opportunities in

other countries. The animal technician is coming to take his place alongside the laboratory technician as an indispensable member of the biological team.

The Committee has already given prominence to the need for national laboratory animal organizations, the four functions of which are: (*a*) to act as an exchange for information about all aspects of animal supply and management; (*b*) to conduct research into current problems in their production and care; (*c*) to maintain primary type colonies of animals conforming to exacting specifications, supplying authentic breeding stock for expansion in production units; (*d*) to train scientists and technicians specializing in this field. For the future, it is planning a second symposium for some time in 1961, on transmissible disease (other than of genetic or nutritional origin); the preparation of world lists of primary type colonies of specified strains of animals, and sources of less commonly used species of vertebrates and invertebrates; as well as continuing the labours already undertaken.

The International Committee on Laboratory Animals started as an informal group, largely self-appointed, to examine a problem that was recognized in many quarters, namely the difficulty in obtaining enough laboratory animals of the right quality for current and future research. It is international in the sense that its membership and interests are already widespread, but it is not yet internationally representative. It is still self-recruiting, and no reason so far exists for it to be otherwise, for its present membership has a strong desire to attract the support of all who share its interests.

The very substantial support so far given by Unesco cannot be expected to continue indefinitely, and the grants given by the international unions are not, and probably never can be, sufficient to cover all its actual and intended activities. If the Committee is to carry on as it has started and to develop usefully, other international governmental agencies should be encouraged to help. Of these, the World Health Organization is an obvious candidate for patronage, because of its involvement in medical research, which is so vitally dependent on good laboratory animals. It is satisfactory to note that the World Health Organization has sent an observer to the last two meetings of the Committee.

It would also be reasonable to expect all countries supporting an active laboratory animals organization to have some representation on the Committee. At present only a few enjoy membership of the Committee, and these would appear to have been somewhat arbitrarily selected. There is little doubt that the lop-sidedness of the Committee, in this respect, will be corrected, and the sooner the better.

There is not only a need, but also great scope for international collaboration in defining and producing good laboratory animals. Without such collaboration there is a danger, already unhappily apparent in many parts of the world, of duplicated effort in solving the major problems in this field; of parallel but isolated developments in the use of animals which appear different but are similar, or appear similar but are differently or misleadingly designated; and of research effort being vitiated by the use of inadequate animal material. This fragmentation of endeavour has to be corrected before it goes too far. By the end of the decade we have just entered, we shall probably consider, in retrospect, our current ideas of quality in laboratory animals with astonishment bordering on incredulity that they served our purposes even as well as they do. If this is so, the International Committee on Laboratory Animals will have played an important part in our animal house revolution.

TRAINING SKILLED WORKERS

ALTHOUGH much is made of the skill of British craftsmen and operators, investigations over the past decade have shown how little is known about the components of skill. The result has been that much of the training provided for manual workers is conducted in a hit-or-miss fashion because those concerned with the training are not at all clear about the nature of the skill they are trying to impart and, consequently, even less clear about the teaching of it. One whose researches have been almost entirely concerned with the teaching of skills to unskilled and semi-skilled operatives in a variety of industries is W. D. Seymour, and, in a broadsheet published by the Institute of Personnel Management, he presents some of the experience he has gained in training unskilled and semi-skilled operatives in manufacturing industry. This broadsheet should be warmly welcomed by all concerned with the training of operatives as the clearest and most concise statement which has yet appeared.

The types of work customarily undertaken by unskilled and semi-skilled workers in manufacturing industry can be grouped into the following categories:

Handwork, that is, work which is done exclusively with the operators' bare hands, without the intervention of any tools or machinery. This group of operations still employs a large number of operators and includes wrapping and packing operations, some assembly work, pottery decoration and other examples of very fine work. Some types of inspection work also fall into this category.

Handwork with Tools. This category includes all types of manual work in which hand tools are used, but which do not involve the use of mechanical power. In spite of the increasing mechanization of modern industry, very large numbers of workers are still engaged in these operations with hand tools, which may be of a general nature, for example, screwdrivers and pliers, or may be specially designed for the purpose, for example, the wig-maker's hook. Examples of this type of work are provided by most types of assembly work, pottery figure making, automatic telephone wiring, coil fitting on electric motors, etc.

Single Purpose Machine Work. This category covers all operations where a mechanically or electrically driven machine is used for a single specific purpose. The increasing use of special-purpose machines tends to augment the number of workers employed in this category, though the numbers employed on any one type of machine may be small. Examples are provided by hosiery seaming and linking machines, coil winding machines and cigar making machines.

Multi-Purpose Machine Work. The category includes all types of work done on adaptable power-driven machines, which can be used for a variety of purposes. Typical examples in this category are the capstan lathe and the industrial sewing machine.

Group Machine Work. This category includes those types of work where the operator is responsible for the output from a number or battery of machines, which are automatic or semi-automatic in their operation. Such work is sometimes referred to as machine minding, but frequently involves short complex operations which need to be done efficiently if full production is to be achieved. Automatic capstan-lathe work, weaving and spinning provide examples in this category.

Non-Repetitive Work. Operations in this category are frequently even more important than repetitive work and involve a high degree of responsibility; they include machine setting, loom overlooking or tackling and inspection operations and process work.

Techniques of operator training are now being used in a number of industries in Great Britain, and are also being adopted overseas. Underlying these techniques is the recognition that operator skills in industry are very varied and highly developed, and that training of new workers to acquire such skills must be based on an adequate analysis and understanding of the skills. Furthermore, training must be considered as continuing right up to the point where the new recruit attains the experienced workers' standard of output and quality. Where the skills analysis indicates that a task presents special difficulties of movement or of perception, exercises or devices are designed for preliminary training. Again, after further scrutiny of the analysis, the task is divided into sections suitable for training, special attention being given to those parts which involve perceptual difficulties or decision making. Target standards, equivalent to experienced workers' standards of output and quality, are determined for each section of the work. Trainees, normally in pairs and in a training centre adjacent to, but separate from, the shop, practise the preliminary exercises, then practise the sections of the job and proceed to perform the parts together only when each has been mastered to experienced workers' standard of output and quality. When one cycle of the operation has been performed at experienced workers' standard, trainees attempt 2-, 4- or 8-hr. runs, passing to a longer period only when targets have been achieved on the preceding shorter period.

By the use of these techniques in a wide variety of industries, such as mechanical engineering, electrical engineering, screw making, pottery, hosiery, garment and footwear manufacture, knitting, spinning and weaving, and other less common industries, it has been found possible to reduce the time required for new entrants to attain the level of performance of experienced workers to between a half and a third of that previously required. Such a reduction in training time, and the higher productivity achieved, lead to economies which usually far exceed the cost of introducing the training scheme. These techniques are also used for the re-training of existing workers whose productivity is below average. The higher output achieved benefits the operators in higher earnings, and the company in lower overhead costs per unit produced.

In the remainder of the broadsheet Seymour describes the methods he has adopted to analyse the nature of skill and some of the findings. Wider use of these findings could do much to increase the quality of skill among British operatives.

This most useful broadsheet may be obtained from the Institute of Personnel Management, 80 Fetter Lane, London, E.C.4, price 7*s.* 6*d.* T. H. Hawkins

OBSERVED CHARACTERISTICS OF AN ULTRA-HIGH-FREQUENCY SIGNAL TRAVERSING AN AURORAL DISTURBANCE

By J. C. JAMES, L. E. BIRD, R. P. INGALLS and M. L. STONE
Lincoln Laboratory, Massachusetts Institute of Technology

J. W. B. DAY and G. E. K. LOCKWOOD
Defence Research Telecommunications Establishment, Canada

AND

R. I. PRESNELL
Stanford Research Institute, Menlo Park, California

An experimental study was made to determine the characteristics of ultra-high-frequency signals propagated through auroral-type disturbances. The experiment was performed by illuminating the Moon from a site in the auroral zone and receiving the reflected signals from the Moon at two receiving sites at mid-latitudes.

The transmitter was located at the Geophysical Institute at College, Alaska. The output power was 10 kW. continuous wave at a frequency of 440 Mc./s. The two receiving sites were located at Shirley Bay, Ontario, near Ottawa, and at the Millstone Hill Field Station at Westford, Massachusetts. Each site used an antenna having two orthogonal polarizations. The signals received were studied carefully to determine whether or not there were any differences when the ionosphere was quiet and when the ionosphere was disturbed by auroral activity.

On at least two occasions a strong auroral disturbance was found in the path to the Moon from the transmitting site at College. On these occasions there were two obvious changes in the characteristics of the received signals: (1) there were rapid fluctuations in the polarization angle; and (2) there was an increase in the rate of fading. However, there was no detectable decrease in average power received during the times of these disturbances.

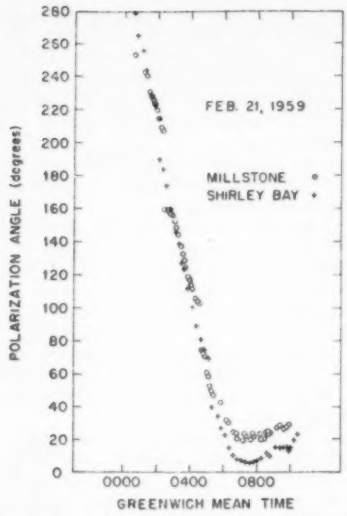

Fig. 1. Variation of the polarization angle of the received signal at the two sites for a day when the ionosphere was quiet

The polarization angle of the signal received was determined by taking the arc tangent of the ratio of voltages in the two orthogonal receiving channels. The quadrant ambiguity resulting from this procedure was resolved by comparing the relative phases of the signals in the two channels before and after a suspected change of quadrant. The polarization angle for a typical lunar day when the ionosphere was quiet is shown in Fig. 1. The polarization angle for this typical run varies slowly with time throughout the day. It was found for these quiet days that the variation of polarization angle could be predicted fairly closely by assuming a certain functional variation of electron density with height, and by adjusting the peak value of this assumed density to match the Central Radio Propagation Laboratory's three-month predictions of '*F*-2 Zero *MUF*'. Account also had to be taken of the relative dipole rotation of the transmitting and receiving antennæ as they tracked the Moon throughout the day with a given polarization.

The measured polarization angles at the two sites for a day when aurora was present are shown in Fig. 2. At about 1400 hr. G.M.T., visual aurora was seen at College in the direction of the Moon. An overcast sky prevented visual observations after about 1500 hr. The rapid fluctuations of polarization angle were apparently caused by the aurora. The computed variation of polarization angle based on a quiet ionosphere is shown by the dashed curve in Fig. 2.

A given change in polarization angle implies a certain change in the number of electrons in the ionosphere, provided certain assumptions are made. One must assume values for the position of the disturbance in electron density and for the magnitude and direction of the magnetic field. On the basis of a stationary magnetic field, the series of fluctuations at 1630 hr. in Fig. 2 represent an incremental change of integrated electron density along a vertical path in the Earth's ionosphere of about 10^{17} electrons per sq. m., assuming that fluctuations in ionization occur below heights of 1,000 km. Very large changes in the direction of the magnetic field are required to explain the observed rapid fluctuations in polarization angle on this basis alone. For example, a change in the direction of the magnetic field of about 20°

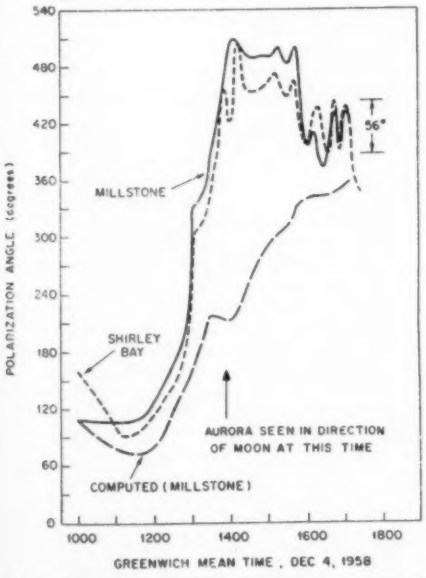

Fig. 2. Variation of the polarization angle of the received signal for a day when the ionosphere was aurorally disturbed. The computed curve is based on a quiet ionosphere

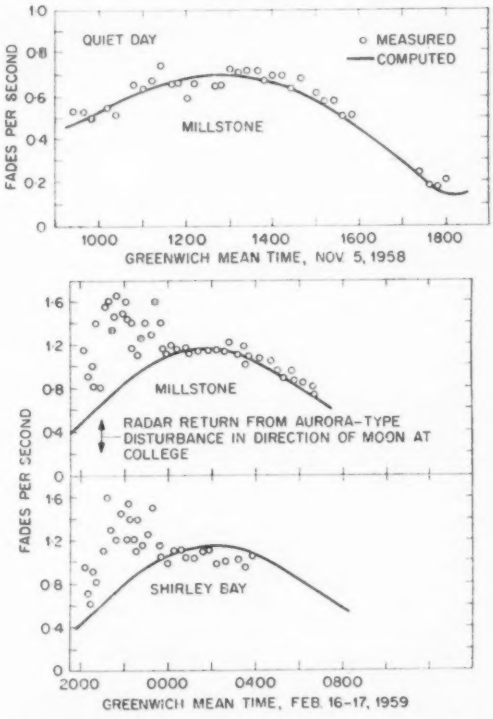

Fig. 3. Comparison of computed and measured rates of fading for a quiet day and an aurorally disturbed day

over a 400-km. height interval in the ionosphere would be required to produce the observed 56° change in polarization angle of Fig. 2 under quiet ionization conditions. This is much larger than any known change in the magnetic field, so most of the rapid fluctuations in polarization angle must be due to rapid changes in ionization density.

During times of an auroral disturbance along the path to the Moon, there was observed an increase in the rate of fading of the received signals. This phenomenon is illustrated in Fig. 3, which shows computed and measured rates of fading for both a quiet day and a disturbed day. During times of a quiet ionosphere, the rate of fading of the received signal was found to be directly proportional to the libration rate of the Moon, an effect that has been reported by others[1]. The predicted rates of fading shown in Fig. 3 were made on the basis of computed rates of libration of the Moon. The top curve illustrates the good agreement obtained between observed and measured rates of fading for normal days. The two lower curves illustrate the increased rates of fading observed during times of auroral disturbances. At approximately 2100 G.M.T. a strong radar return was observed at College from an auroral disturbance in the direction of the Moon. The measured rate of fading at this time was considerably larger than the predicted value made on the basis of Moon libration.

In summary, the significant results reported here are that an auroral disturbance in the path of propagation causes a rapid fluctuation in the polarization angle of the signal received, and an increase in the rate of fading of the signal received, but no measurable absorption of the signal itself. The observed rapid changes in polarization angle are probably due to changes of electron content of the ionosphere of the order of 10^{17} electrons in a vertical column of 1 sq. m. cross-section.

This experiment was a co-operative programme by the following three laboratories: (1) Lincoln Laboratory, a centre for research operated by the Massachusetts Institute of Technology with the joint support of the U.S. Army, Navy and Air Force; (2) Stanford Research Institute, the work of which was sponsored by the Rome Air Development Center of the Air Research and Development Command under Contract *AF* 30 (602)-1762; and (3) the Defence Research Telecommunications Establishment of the Defence Research Board of Canada, the work of which was performed under Project No. *PCC D*48-38-01-11.

We wish to acknowledge the contributions made by our associates at the Lincoln Laboratory, at the Defence Research Telecommunications Establishment, and at the Stanford Research Institute throughout the course of the experiment. We are also grateful for the assistance furnished by personnel of the Geophysical Institute and of the Coast and Geodetic Survey Laboratory at College, Alaska.

[1] See, for example, Kerr, F. J., and Shain, C. A., *Proc. Inst. Rad. Eng.*, **39**, 230 (1951). Browne, I. C., Evans, J. V., Hargreaves, J. K., and Murray, W. A. S., *Proc. Phys. Soc.*, **69***B*, 901 (1956).

OXIDATION OF HYDROXYLAMINE IN CELL-FREE EXTRACTS OF *NITROSOMONAS EUROPAEA*

By Dr. D. J. D. NICHOLAS* and Dr. O. T. G. JONES*

Long Ashton Research Station, University of Bristol

We have prepared cell-free extracts of *Nitrosomonas* that will oxidize hydroxylamine to nitrite provided a suitable acceptor such as cytochrome *c* or phenazine methosulphate is added to the extract or to protein fractions prepared from it. Since this is probably the first report of a cell-free system capable of oxidizing hydroxylamine, we give here a brief summary of our main findings.

Cultures of *Nitrosomonas europaea* (kindly supplied by Dr. Jane Meiklejohn, of Rothamsted Experimental Station) were grown in a culture solution based on that used by Meiklejohn[1] and later modified by Walker (private communication): $(NH_4)_2SO_4$, 0·66 gm.; NaCl, 0·2 gm.; K_2HPO_4, 0·13 gm.; $MgSO_4.7H_2O$, 0·24 gm.; $FeSO_4.7H_2O$, 0·5 gm. adjusted with 5 per cent w/v K_2CO_3 to *p*H 8·5, glass-distilled water to 1 litre). Phenol red was used as an internal indicator and the *p*H of the medium was maintained constant by titrating it continuously with sterile 10 per cent w/v potassium carbonate. Batch cultures of bacteria (300 l.) were grown at the Medical Research Council Antibiotics Research Station, Clevedon, through the courtesy of the director, Mr. B. K. Kelly, and with the help of Dr. R. W. Harrison; their assistance is gratefully acknowledged.

The bacteria suspended in 0·01 *M* phosphate and 0·05 *M* sodium borate buffer (*p*H 8·3) were disintegrated by means of a Mullard ultrasonic probe for 15 min. at 4° C. in a double-walled glass vessel through which iced water was circulated. The homogenate was centrifuged at 20,000*g* for 20 min. at 4° C.; the supernatant solution was used as the source of the crude enzyme and for subsequent fractionation. Enzyme activity was followed by measuring nitrite production from hydroxylamine in the following reaction mixture: 0·1 ml. enzyme, 0·1 μmole hydroxylamine hydrochloride, 0·2 μmole cytochrome *c* (95 per cent pure, Sigma Chemical Company, U.S.A.) or 0·2 μmole phenazine methosulphate contained in 1 ml. of the phosphate–borate buffer (*p*H 8·3). After the stated incubation period (15 or 20 min.), 0·5 ml. of 1 per cent w/v sulphanilamide in *N* hydrochloric acid and 1 ml. of 0·01 per cent w/v N-(1-naphthyl)ethylenediamine dihydrochloride were added and the volume made to 5 ml. with distilled water, and nitrite determined in a 'Spekker' absorptiometer. Enzyme activity was also determined by following oxygen uptake in a Warburg

* Members of the scientific staff of the Agricultural Research Council seconded to Long Ashton Research Station, University of Bristol; Dr. O. T. G. Jones is now at the Biochemical Department, Fisons Pest Control, Chesterford Park, Saffron Walden, Essex.

Table 1. Effects of Anions on Nitrite Formation from Hydroxylamine in Cell-free Extracts of *Nitrosomonas europaea* using Cytochrome *c* as the Acceptor

	Citrate	Selenate	Arsenate	Tungstate	Phosphate	Pyrophosphate
Per cent inhibition	93	93	85	95	84	90

Silicate and tellurate had no effect.
Reaction mixture: 50 μmoles of the anion, 0·1 μmole cytochrome *c*, 0·1 μmole hydroxylamine hydrochloride in a final volume of 1 ml., phosphate–borate buffer (*p*H 8·3), incubated for 20 min. at 30° C.
Nitrite assayed as in text.

Table 2. Fractionation of the Enzyme System in Cell-free Extracts of *Nitrosomonas europaea* that Oxidizes Hydroxylamine to Nitrite using Phenazine Methosulphate as the Acceptor

Fraction	Volume (ml.)	Total units (mμ*M* nitrite/20 min.)	Total protein (mgm.)	Specific activity (mμ*M* nitrite/20 min./mgm. protein)	Purification (fold)	Percentage recovery of enzyme
(1) Crude extract after centrifuging at 20,000*g* for 20 min.	20	6,500	37·4	192		
(2) Fraction collected between 80 and 90 per cent saturation with ammonium sulphate dissolved in 0·01 *M* phosphate and 0·005 *M* borate buffer (*p*H 8·4)	10	2,850	1·1	5,200	27	44
(3) 5 ml. aliquots of (2) put on a *D.E.A.E.*-cellulose column (15 cm. × 1·2 cm.). Column washed in turn with 10-ml. aliquots of the following buffers (*p*H 8·5): 0·05 *M*, 0·1 *M* phosphate-borate; 0·25 *M*, 0·5 *M* phosphate and finally 0·2 *M* pyrophosphate						
Activity of pyrophosphate eluate	5	510	0·2	8,500	44	8

apparatus in the presence of phenazine methosulphate or cytochrome *c*.

The enzyme activity, which was linear with time when either phenazine methosulphate or cytochrome *c* was used as acceptor, has a *p*H optimum between 7·0 and 8·6. Maximum activity was obtained with 0·2 μmole of either acceptor per 0·1 ml. crude extract containing about 1 mgm. protein per ml. Phenazine methosulphate was the best acceptor, and pyocyanine, methylene blue, benzyl viologen or cytochrome *c* were usually less active, whereas ferricyanide, 2.3.6.-trichloroindophenol dye, coenzymes I and II or glutathione had little effect. Similar results were obtained with whole cells.

When cytochrome *c* was the acceptor, phosphate was required, but at higher concentrations the anion inhibited the oxidation of hydroxylamine as shown in Fig. 1. The phosphate inhibition, which was competitive for cytochrome *c* as shown in Fig. 2, did not occur with phenazine methosulphate. The extent of the inhibition by various anions including pyrophosphate, citrate, tungstate, selenate and arsenate is shown in Table 1. Similar results were

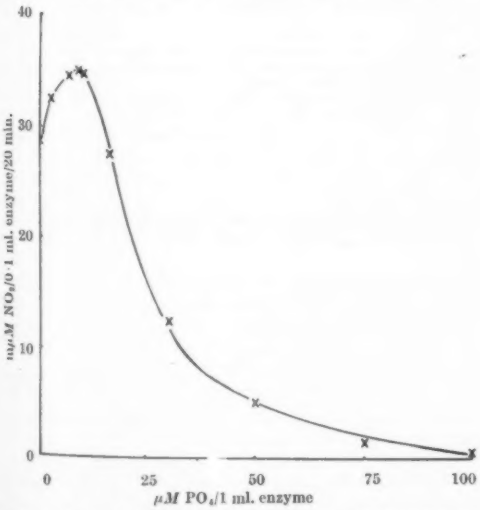

Fig. 1. Effect of phosphate on the oxidation of hydroxylamine to nitrite in a crude extract of *Nitrosomonas europaea*, well dialysed for 12 hr. against 0·01 *M tris* (hydroxymethyl) amino methane buffer, *p*H 8·5
Reaction mixture: 10 μmoles *tris* buffer (*p*H 8·5); 0·2 μmole cytochrome *c*, 0·1 μmole hydroxylamine hydrochloride, 0·1 ml. enzyme contained in 1 ml. incubated for 20 min. at 30° C.
Nitrite determined as described in text

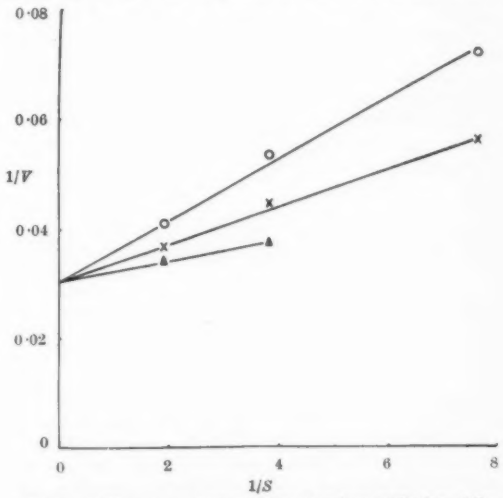

Fig. 2. A Lineweaver–Burk plot for phosphate inhibition of the oxidation of hydroxylamine to nitrite in crude extracts of *Nitrosomonas europaea*, dialysed as described under Fig. 1, using cytochrome *c* as the acceptor
Reaction mixture: 10 μmoles *tris* buffer (*p*H 8·5), 0·1 μmole hydroxylamine hydrochloride, 0·1 ml. enzyme and cytochrome *c* as indicated, contained in 1 ml. incubated for 20 min. at 30° C.
Nitrite determined as described in text
1/*S*, reciprocal of cytochrome *c* concentration in mgm. per reaction mixture; 1/*V*, reciprocal of mμ*M* nitrite formed/0·1 ml. enzyme/20 min.

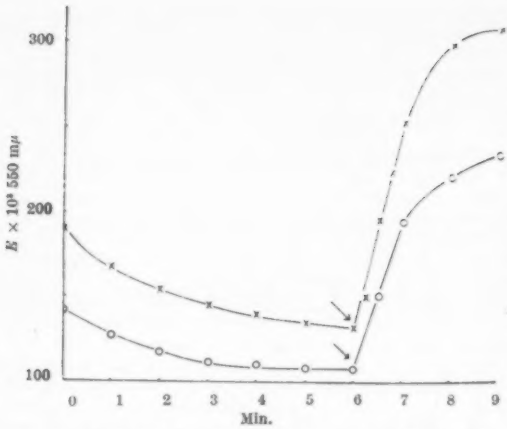

Fig. 3. Effect of hydroxylamine and hydrazine on the enzymic reduction of cytochrome *c* in cell-free extracts of *Nitrosomonas europaea*

+——+, ↓, addition of 0·2 μmole hydroxylamine hydrochloride; ○——○, ↓, addition of 0·2 μmole hydrazine

Reaction mixture in a 1-cm. spectrophotometer cell: 25 μmoles phosphate, 5 μmoles sodium borate buffer (*p*H 8·5), 0·05 μmole cytochrome *c*, in a final volume of 3 ml. and enzymic oxidation of cytochrome *c* allowed to go to completion, then 0·2 μmole of hydroxylamine or hydrazine added

Reaction followed in a Unicam *S.P.*500 spectrophotometer at 550 mμ. Hyponitrite (0·2 μmole), when added instead of hydroxylamine, had no effect. No oxidation or reduction of cytochrome *c* was observed with a boiled enzyme

obtained with whole cells. Hydrazine, a well-known inhibitor of nitrite formation from hydroxylamine in washed cells of *Nitrosomonas*[2], had a similar effect on the cell-free system when cytochrome *c* was the acceptor; but there was no inhibition with phenazine methosulphate. The results in Fig. 3 show that there was an active cytochrome oxidase in the extracts, and the subsequent addition of hydroxylamine resulted in an immediate enzymic reduction of cytochrome *c* with the formation of nitrite. The addition of hydrazine instead of hydroxylamine produced an enzymic reduction of cytochrome *c*, but nitrite was not produced. An increase in hydroxylamine concentration decreased the inhibition of nitrite production by hydrazine. Thus the hydrazine inhibition of nitrite formation in whole cells may be due to a competition with hydroxylamine for a common acceptor such as cytochrome *c* which is indispensable for the oxidation of hydroxylamine. Cyanide inhibited when a range of acceptors was used but carbon monoxide, although depressing cytochrome oxidase activity, did not reduce the oxidation of hydroxylamine to nitrite.

Neither hyponitrite nor ammonia alone was oxidized by these extracts, but the addition of ammonia markedly stimulated the oxidation of hydroxylamine; for example, addition of ammonium sulphate to the reaction mixture at five times the concentration of hydroxylamine stimulated nitrite production by about 60 per cent. Another interesting phenomenon is that the nitrite formed from hydroxylamine in these extracts using cytochrome *c* or phenazine is only 40–73 per cent of the theoretical values expected from the oxygen uptake results obtained in the Warburg. The hydroxylamine was completely oxidized, as confirmed by analysis[3], in agreement with the oxygen uptake. Oxidation with iodine, boiling or acid treatment before the nitrite test did not increase nitrite. When the extracts were aged in the refrigerator for a week or so the nitrite values in the enzyme assays increased, but at best they only approximated to 73 per cent of the theoretical amounts.

A forty-four-fold purification of the enzyme system was achieved by precipitation with ammonium sulphate and further fractionation on diethylaminoethyl-cellulose columns. The pyrophosphate eluate of this column contains the active preparation, using phenazine methosulphate, since pyrophosphate inhibits when cytochrome *c* is the acceptor. The final fraction may contain less protein than that recorded, since pyrophosphate elutes non-protein material from the diethylaminoethyl–cellulose column which reacts with the Folin protein reagents and also absorbs at 260 and 280 mμ.

A more detailed account of this work is in preparation.

[1] Meiklejohn, J., *J. Gen. Microbiol.*, **8**, 58 (1953).
[2] Lees, H., "Biochemistry of Autotrophic Bacteria" (Butterworths Publications, London, 1955).
[3] Frear, D. S., and Burrell, R. C., *Anal. Chem.*, **27**, 1664 (1955).

RESPONSE OF INBRED AND F_1 HYBRID MICE TO HORMONE

By Dr C. K. CHAI

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

IN the hands of the biologists the house-mouse is one of the mammals subjected for a long time to continuous mating between close relatives, thereby having attained a high degree of inbreeding and the greatest number of inbred lines with distinct biological characteristics. Because of its biological and genetic uniqueness, and its high rate of reproduction and ease of maintenance, this small-sized mammal has gained recognition as an experimental animal of great value in genetic and medical research and in biological assay. It is on the use of the mouse in bioassays that questions have been raised as to whether inbred mice are superior to random-bred mice and to animals of equal genetic homogeneity but heterozygous, such as F_1 hybrids, produced by crossing two inbred strains.

About two decades ago Emmens[1] found that some F_1 hybrid mice were less variable in response to œstrogen than their inbred parental strains. Later, Mather[2] presented some genetic considerations involved in biological responses. Recently, some data have been presented by McLaren and Michie[3], Claringbold and Biggers[4], and McLaren and Michie[5], and a brief review has been made by Biggers, McLaren

and Michie[6] concerning the suitability of using inbred animals as compared with F_1 hybrid animals and random-bred stocks in biological assays. It has been claimed by the latter that inbred animals are not desirable for bioassay on the grounds that they are more variable in responses than either the F_1 hybrid or random-bred animals.

The problem concerning the choice of assay animals requires further consideration. Biological responses involve the reactions in the animal body of one to many systems, all of which are controlled to different extents by the genetic constitution of the individual. The nature of the responses is complicated, and the end-points used are different from one assay to another. However, in determining the precision of an assay, an important parameter which must be considered in quantitative assays—in addition to variance—is sensitivity of response as measured by the slope of the dose–response curve.

This article, which presents some of my experimental results and reviews briefly the results published by others, concerns mainly the sensitivity of response to hormonal substances based on the principles of bioassay and experimental evidence from mice. Some genetic interpretations and general conclusions are made. Although the evidence is drawn from experiments with hormones as assay substances and mice as assay animals, the conclusions reached may be applicable to other drugs and biological preparations and other animal species.

Requirements of an Assay Animal

Biological assays may be defined as determinations of potency or toxicity of a given substance based upon the response of living matter. The nature of the response may be: (1) alteration of internal state—adjustment; or (2) maintenance of relative constancy of the internal state—regulation. It is very likely that most biological stimuli cause both mechanisms to operate at the same time. For convenience of measurement, biological responses are ordinarily classified into two main categories: (1) qualitative or all-or-none response; and (2) quantitative. A qualitative response is measured by the percentage of assay subjects with a positive reaction. The most common qualitative data are quantal. An alternative method for quantal data is the use of reaction time, which is relatively seldom used in hormone assays. A quantitative response is very commonly used in hormone assays; it should give more information per observation than a quantal reaction.

The design of the assay and its computation depend mainly upon the characteristics of the response to be considered. The basic principles for satisfactory assay by Gaddum[7] may be adapted as a guide in the choice of an assay animal. These principles are: (1) a linear relationship should exist between response and dose over the widest possible range of dose; (2) the standard deviation should be independent of the response; (3) a minimal value for the ratio s^2/b^2 in quantitative assay and $1/b^2$ in quantal assay, where s^2 is the within-group variance and b is the slope of the dose-response line. (This ratio is called λ^2 by Gaddum.) In addition, good assay animals should give reproducible results, and the precision of an assay should not alter much from test to test or from time to time.

The answer to the question as to whether F_1 hybrids are preferable to inbreds in hormone assay can be derived from calculations based on the formula $\lambda = s/b$ in quantitative assay and $\lambda = 1/b$ in quantal assay. Inbred strains, or F_1 hybrids, which give the smallest λ, offer the most precise assay information or the most information per animal.

In quantitative response, comparison of the assay value between the inbreds and F_1 hybrid animals is therefore based on the ratio of s to b. The ratio may not be minimal when s is minimal, since it depends on the relationship between the two quantities. A slope which is steep enough may compensate for a large variance. Thus, the choice of a mouse strain or F_1 hybrid for bioassay of a given substance is governed by the two parameters in quantitative assay, rather than by either one alone.

In assays involving the quantal response, the precision of the assays depends entirely on b. The value of b, in fact, measures the variation in response of the particular group of animals, for the variances are entirely dependent on b, since $s^2 = 1/b^2$. Unless reliable information is available, the choice of optimum dose-level and adequacy of transformation is frequently a troublesome matter in practice, especially in comparisons of genetically different groups of animals. Inadequate transformations or unsuitable dose-levels easily lead to biased estimates of the true value of b, which in turn results in biased estimations of variance. For this reason the quantitative responses are considered to be more appropriate than the quantal responses in comparing the bioassay value between genetically different groups of animals.

Difference in Sensitivity or Magnitude of Response

Sensitivity is measured by the slope of the dose–response curve. Magnitude of response at a given dose may also be indicative of sensitivity but only when comparing dose–response curves which (1) originate from approximately the same point, and (2) are linear after appropriate transformation. The following examples cover mice of many inbred strains and F_1 hybrids popularly used in bioassays of various hormones. Some of my unpublished results are given first and followed with a tabulation of results published from this and other laboratories.

Response to Androgen. Mice of the *C57BL/6* and *C57BR/cd* inbred strains and their F_1 hybrid BBF_1 were used in this experiment. (The two inbred strains were comparatively closely related and their biological characteristics are rather similar.) The mice were weaned at about four weeks of age, and only those of 9–12 gm. body-weight were used. They were castrated within three days after weaning. Animals were divided into dose groups (eight animals each) in each strain and in the F_1 hybrid mice, and each dose group received a different dose of injection of testosterone in liquid suspension. The injection was begun on the day following castration, one dose a day, and continued for twenty days. The mice were weighed and autopsied on the day following the last injection. The seminal vesicles were removed, blotted thoroughly, and weighed. The means and variances of seminal vesicle weights in each dose group are given in Table 1 and illustrated in Fig. 1. (The methods were similar to a previous experiment using testosterone propionate in different inbred strains of mice—for details see Chai[8].) There

Table 1. Means and Variances of Seminal Vesicle Weights in response to Testosterone in Inbred and F_1 Hybrid Mice

Dose (mgm.)	C57BL/6 mean (mgm.)	C57BL/6 s^2	C57BR/cd mean	C57BR/cd s^2	F_1 (C57BL/6 × C57BR/cd) mean	F_1 (C57BL/6 × C57BR/cd) s^2
0·016	1·9	0·19	2·9	1·33	1·5	0·15
0·032	2·3	1·09	4·4	1·33	3·4	0·58
0·064	6·6	1·10	12·5	4·41	4·7	1·71
0·128	20·3	6·39	24·4	7·25	19·7	16·79
0·256	26·9	35·63	28·8	19·10	29·8	4·97

appears to be no difference in the slope of the dose–response curves between the three different groups of mice. The F_1 hybrid was no more sensitive than either one of the parental strains in this particular response.

Response to Gonadotrophin. (The following forms a part of the results of an experiment carried out by J. Leopold, as a summer student under my guidance.) Female mice about one month of age from inbred strains, *DBA*/1, *DBA*/2, *BALB*/*c*, *A* and the F_1 hybrid of the last-named two strains, were divided into dose groups. Chorionic gonadotrophin was serially diluted. One dose was assigned to each group and two injections were given to each mouse at 24-hr. intervals. Autopsy was made at about 12 hr. after the last injection. The presence of hæmorrhagic follicles in the ovaries was considered a positive response. The percentage of animals giving a positive response was calculated for each dose group. Probit transformation was made, and a linear regression was computed for each strain and hybrid according to the methods described by Finney[9]. The results are given in Table 2. The regression of the dose response appears to be largest for the *BALB*/*c* and least for the *A* strain; that of the F_1 hybrid fell between those of the parental mouse strains but close to that of the *A* strain. The difference in slope between the two *DBA* sublines, though not large, seems to show their genetic differences in the response as a consequence of separation in breeding for about twenty generations.

Table 2. Regression of Response in Percentage of Ovarian Follicles transformed to Probits on Serial Doses of HCG in Inbred and F_1 Hybrid Mice

Genetic groups	No. mice	Regression equations* $(a + bx)$
DBA/1	80	$4·91 + 1·25x$
DBA/2	80	$4·96 + 0·99x$
A	88	$4·69 + 0·62x$
CAF_1	80	$5·19 + 0·64x$
BALB/*c*	70	$5·61 + 1·27x$

* a, fitted constant; b, slope; x, log dose.

The published results using various hormones as sources of stimulation are given in Table 3. In addition to the differences in responses shown among inbred strains, out of nineteen comparisons of responses between F_1 hybrid mice and their parental inbred strains, ten hybrids fell between their parental strains, four were comparable to, and five greater than, either one of the parents.

Genetic Basis of Hormonal Response

A biological response, either physiological or morphological, varies within limits set by the genotype, although it is influenced greatly by environment. This is clearly indicated by: (1) the differences in response to various hormones between the inbred strains in the given examples; (2) response to selection for sensitivity to gonadotrophin[22]; (3) relative proportion of genetic to environmental variance in gonadotrophin assay[23]; (4) the heritability of iodine metabolism in the thyroids of mice[24], and the difference in response to thyroxine between inbred strains

Table 3. Relative Sensitivity or Magnitude of Response to Hormones in Inbred and F_1 Hybrid Mice

Hormone applied	Criterion of response	Relative sensitivity* or magnitude of response	Author
Œstrogen	Vaginal smear	$C_{57} > C_{52} \times CBA > CBA$ $C_{52} > CBA \times C_{57} > CBA$ $CBA \sim CBA \times A \sim A$	Trentin (ref. 10)
Œstrogen	Vaginal smear	$C_{57}BL > C3H \sim C$	Shimpkin (ref. 11)
Œstrone Intravaginal	Vaginal smear	$C_{57} \times CBA \sim CBA \times C_{57} > CBA > C_{57}$	Claringbold and Biggers (ref. 4)
Œstrone-3 : 17β Subcutaneous		$C_{57} \times CBA < CBA \times C_{57} > CBA$	
Œstrone and pregesterone	Mammary gland area	Castrate male $C3H > A > BALB/c > C57BL$ $BALB/c > BALB/c \times A > A$ $BALB/c > A \times BALB/c$ $C3H > C3H \times C57BL > C57BL \times C3H > C57BL$	Blair (ref. 12)
Mammary-gland stimulating hormone	Mammary lobule alveolar growth	Schwing > Swiss > Sutter ~ Kansas	Mixner and Turner (ref. 13)
Œstrogen	Adrenal weight (per cent of control)	Both ♂ and ♀ $BALB/c > BALB/c \times C3H > C3H/He$ $BALB/c > BALB/c \times A > A/He$ $C_{57}BL > C57BL \times C3H > C3H$ $C_{57}BL \sim C3H \times C_{44}BL > C3H$	Westberg (ref. 14)
Testosterone propionate	Kidney *B*-glucuronides	$A/Cl > AKR > BAF_1 > C3H > CAF_1 > DBA > 129$	Fishman and Farmelant (ref. 15)
Cortisone	Number of eosinophils	$129 \times BR/cd > C57BL \times C57BR/cd > C57BL/6 > C57BR/cd > ST > C57BR/a > 129/Rr > DBA > BALB/c$	Wragg and Speirs (ref. 16)
Chorionic hormone	?	$C57 > A$	Carr (ref. 17)
Œsrone	Mammary growth	$C57Bl \sim O_{20} > DBA$	Mühlbock (ref. 18)
	Œstrus symptoms	$C57BL \sim O_{20} =$ Riet $> DBA$	
Œstrone	Mammary growth	(Castrate male) $DBA > C57BL \sim O_{20} >$ Riet	Mühlbock (ref. 19)
	Per cent vaginal œstrus	$C57BL \sim O_{20} > DBA$	
Human pregnancy urine	Per cent ovarian follicles	$C57BL \sim C57L > A$	Hummel (ref. 20)
Testosterone propionate	Seminal vesicle weight	$BALB/c > CAF_1 > A/Jax$	Chai (ref. 8)
PTU and thyroxine	Thyroid weight	$A/Jax > BAF_1 > C57BL/6$	Chai (ref. 21)
	Thyroid follicular cell height	$DBA/1 \sim DBA/2 \sim BDF_1 > C57BL/6$ $BAF_1 > C57BL/6 \sim A/Jax$ $BDF_1 > DBA/_1 > DBA/2 > C57BL/6$	

Remarks: $BAF_1 = C57BL/6 \times A/Jax$; $CAF_1 = BALB/c \times A/Jax$; $BDF_1 = C57BL/6 \times DBA/2$

* The sequence is based mainly on observations of graphic illustrations and/or the mean values of responses. The differences between strains and F_1 hybrids in a response as given may not be statistically significant and the original authors might not fully agree with the given order.

of mice and their F_1 hybrids as determined by iodine turnover rate in the thyroids[25].

It may be pointed out particularly that on the results concerning the responses of *BALB/c* and *A* strains and their F_1 hybrids (Table 3), one is the response to œstrone and progesterone, measured by mammary gland area[13], and the other is response to œstrogen as measured by adrenal weight[14]. In both experiments, the F_1 hybrid fell between the parental strains and the order is the same as in assays using androgen[7] and gonadotrophin for stimulation. These results are from different laboratories, but in spite of possible differences in environmental conditions and in assay techniques, the same order of relative sensitivity has been obtained in mouse stocks. This may indicate that the same, or closely linked, genes control some of the related endocrine systems, and contributes further evidence toward the genetic determination of hormonal responses.

Except in a few cases (such as resistance to insulin in obese mice and responsiveness to pituitary extract in dwarf mice) where the action of a single gene can have a significant effect upon certain responses, the inheritance units determining hormonal responses in the mice commonly used in bioassays must be assumed at present to be polygenes. The biological properties of these polygenes may be considered to be the same as those which determine characters generally used in quantitative genetic studies. That is, their effects are small, additive and supplementary. The additive effects of polygenes in general have been found to be more important than dominance and epistatic effects, if the latter exist.

On this basis, the consequence in hormonal responses of F_1 hybrids in comparison to their parental strains may be tentatively postulated. When the parental strains differ greatly in a given biological response, it may be assumed that one strain possesses most of the alleles with plus effect and the other parent alleles with minus effects in the loci concerned. The consequence of combining one from each pair of alleles of these two sets as in the F_1 hybrids would result in an intermediate response or one close to it. It is supposed that in general the dominant effects are weaker than additive effects in polygenic inheritance. Therefore, even the net effects of dominance may not be exactly cancelled but the response in the F_1 will be unlikely to exceed those of either one or the other parental strain in the response concerned. This supposition seems to hold in many animal experiments dealing with quantitative characters and may hold in the present case. On the other hand, when the parental strains are similar in response, it is possible for the F_1 hybrids sometimes to give responses either greater or smaller than either parental strain, as a result of the supplementary effects or interaction of genes with the other genetic background. The responses to testosterone in four inbred strains of mice and two F_1 hybrids (Figs. 1 and 2), and results published by various investigators as given in Table 3, seem, in general, to support the above hypothesis. It is realized that this is a great over-simplification in this brief postulation of the genetic basis of hormonal responses, but, regrettably, until better understanding of the genetic and physiological properties of polygenes are arrived at, further speculation would be impractical.

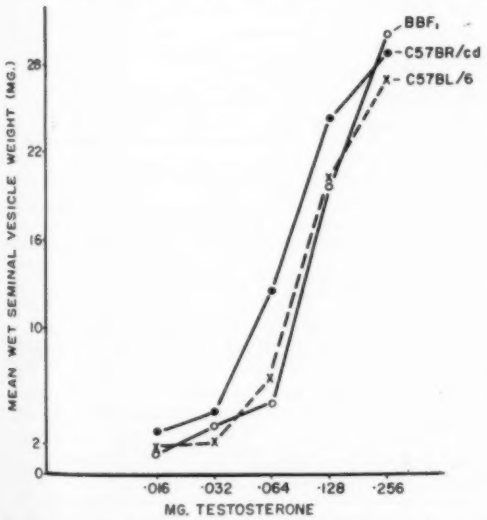

Fig. 1. Showing the response to graded doses of testosterone as measured by wet seminal vesicle weights in *C57BL/6*, *C57BR/cd* and their F_1 hybrid mice, BBF_1. It appears that there are no differences between them in sensitivity

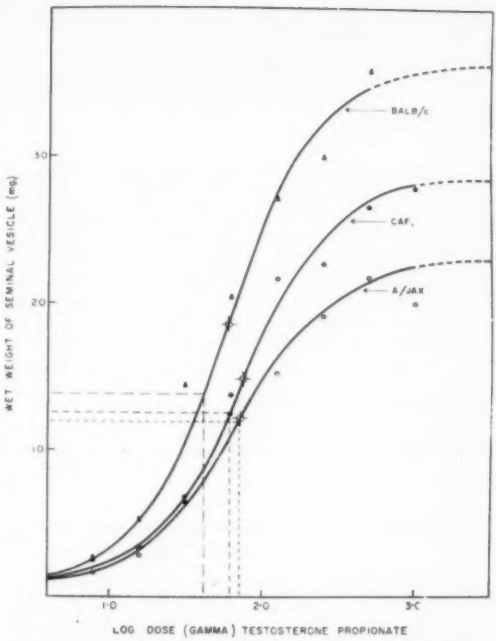

Fig. 2. Showing the distribution of seminal vesicle weights and fitted logistic curves for the *A/Jax*, *BALB/c* strains of mice and their F_1 hybrids treated with graded doses of testosterone propionate. The slope of the dose-response curve of *BALB/c* mice is greater than that of *A/Jax*, and that of their F_1 hybrid is about intermediate between those of the parental strains (from Chai, ref. 8, courtesy of *Anat. Rec.*)

Conclusions

The above data show that in the majority of cases the responses of the F_1 hybrids to various hormone stimulations fell between those of their parental strains. This indicates that the F_1 hybrids are not, in general, preferable to inbred mice in hormone assay in so far as the *b* value is concerned, for *b* is one of the two parameters in quantitative response and

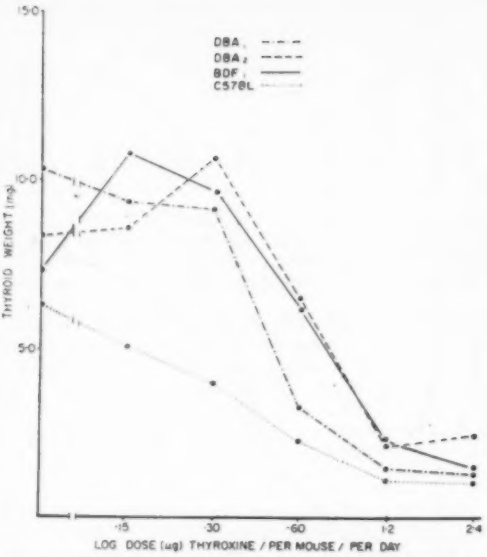

Fig. 3. Distribution of mean thyroid weights in the mice of *C57BL*/6, *DBA*/1, *DBA*/2 and BAF_1 (*C57BL*/6 × *DBA*/2). Each point represents a mean thyroid weight at a given dose from eight mice. Each mouse received a subcutaneous injection of thyroxine, one dose a day, for twenty days. All mice were on a diet of Purina Mouse Laboratory Chow mixed with 0·2 per cent of propylthiouracil during the treatment. Note the long linear portion of the curve for *C57BL* mice, which is desirable in bioassay, although the slope of the curve is smaller than that of the curves for the *DBA*'s and the BAF_1 (from Chai, ref. 21 ; courtesy of *J. Heredity*)

the only parameter in qualitative response in determining the precision of the assay.

The linear relationship between response and dose over the widest possible range of dose is another matter worthy of consideration. It is noticed that the response to both goitrogen and thyroxine in the *C57BL* mice was distributed linearly throughout practically all the dosages applied, whereas the linear portion for the F_1 hybrids fell within a limited range of dose[21] (Fig. 3). Whether this is due to better self-regulating properties of the F_1 hybrids, such that they can use the hormones more efficiently, is a problem remaining for further exploration.

It is generally claimed that F_1 hybrids are more uniform in growth and development than inbreds and this is, no doubt, a desirable feature in bioassay. The importance of body-size differences affecting the response depends on the degree of relationship between the criteria of response and body-size. In most cases, this can be handled satisfactorily by covariance analysis. The part of the variation in response as caused by differences in body-size should not be interpreted as the intrinsic difference in magnitude of reactivity of the assay animals themselves, but represents nothing more than the phenotypic heterogeneity of the assay subjects. More extensive data are needed to prove differences between the two types of genetically homogeneous populations, allowing for body-size differences. That is, whether there are basic differences in variation of reactivity to stimulation, which have not resulted from pre-existing anatomical or physiological differences in variability, remains to be studied.

It has been suggested that under unfavourable environmental conditions the homozygotes are more variable than the heterozygotes in growth and development. This might apply to biological responses and explain the differences obtained by investigators from different laboratories and the so-called transient effect in some laboratories. It is possible that the F_1 hybrids may stand long-distance shipment and change of environment better than inbreds, due to their better self-regulating ability and resistance to disease. This is a matter which needs to be considered in practice. On the same argument, it may be true that the homozygotes, such as the inbred mice, can be more subject to internal environmental changes of physiology and thus show more sensitive responses in bioassay than do heterozygotes. The recent report by Becker *et al.*[28] comparing growth response of inbred and non-inbred chickens at different nutritional levels seems to support this hypothesis.

In summary, F_1 hybrids do not necessarily have greater sensitivity as measured by the magnitude of responses at each dose-level. That F_1 hybrids are, in general, more uniform in growth and development and better adjusted to environmental changes than inbreds is often claimed. This is expected to affect the variability of response and may be an advantage of F_1 hybrids over inbreds in bioassays. Depending on the genetic background, some inbreds or F_1 hybrids may be more suitable for certain hormone assays than for others, and animals of some genotypes may be less variable in one environment than in another. For the assay of a given substance, the choice of assay animal—either an inbred or an F_1 hybrid—cannot be made in advance ; it has to be based either on existing data or determined experimentally.

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LETTERS TO THE EDITORS

GEO- and RADIO-PHYSICS

A Day-time Maximum of Oblique Auroral Reflexions observed in the Auroral Zone

Almost all investigations of high-frequency and very high-frequency reflexions from aurora made in or near the auroral zone have given diurnal variation curves with one main maximum near local midnight[1–3]. An exception is the recent observations by Presnell *et al.*[4] at 216 Mc./s., made at College, which—for diffuse echoes only—has given two maxima, one in the morning and one in the afternoon with the peak between 1700 and 1900 L.T. Observers at lower latitudes have usually found two maxima in the night[5–8].

From four months records (March–June 1959) of aurorally propagated very high-frequency signals taken in the auroral zone at Kiruna Geophysical Observatory (geographic co-ordinates 67·8° N., 20·4° E., geomagnetic co-ordinates 65·3° N., 115·5° E.) and transmitted by the Finnish broadcasting station at Kemi (geographic co-ordinates 65·8° N., 24·8° E., geomagnetic co-ordinates 62·5° N., 117·6° E.) a diurnal variation curve with two maxima has been found (cf. Fig. 1). One of these maxima is in day-time between 1200 and 1600 M.E.T. The experimental points include all observed reflexions. This is in remarkable contrast to the diurnal variation mentioned above, which was observed by radar methods at Kiruna (in 1951–52) and at other places in auroral zone latitudes.

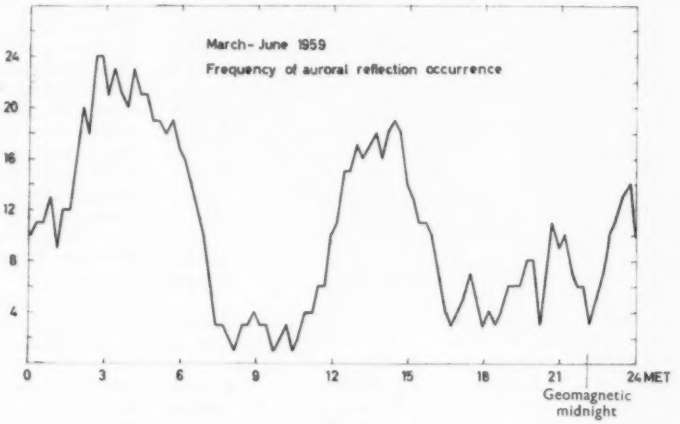

Fig. 1

The transmissions studied at Kiruna have a frequency of 92·8 Mc./s. The broadcasting station, 310 km. distant, has sent out continuous waves in the periods between programme transmissions. The radiated power is 8·9 kW. and the polarization horizontal. A 'Servo' model *R* 5200-*A*2 receiver and a five-element Yagi antenna are used. The sensitivity is better than 1 μV. for 50 mW. output for a bandwidth of 25 kc./s.

There is no doubt that this auroral echo day-time maximum is real. The day-time echoes have the same signal characteristics observable[2] as night echoes[1] on the Esterline Angus records. The direction distributions for day- and night-maxima are approximately the same; this has been determined by means of rotary antennæ. The day-time maximum has been found also in monitoring another transmitter (Östersund in central Sweden, 87·9 Mc./s.).

The local time for the afternoon echo maximum of Fig. 1 is much earlier than that found by any other observers, so far as we are aware. If the geomagnetic time for the maximum is investigated, the difference from the time of maximum found on the western hemisphere (especially in Alaska) is reduced by several hours, but also the geomagnetic time for the afternoon maximum found at Kiruna is earlier than reported by anybody else. Recent work at a frequency of 216 Mc./s. (ref. 4) has given an afternoon maximum, but only for a special type of echo, the so-called diffuse echoes. The difference between the geomagnetic time for the afternoon maximum reported by Presnell *et al.*[4] and that shown in Fig. 1 is about 1 hr.

The investigation at Kiruna will be extended over a longer period of time, and it is hoped that the recorded material will show if the diurnal variation curve described above is characteristic of observations of oblique auroral very high-frequency reflexions at Kiruna for longer periods, or only for the four months analysed.

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Alv Egeland
Bengt Hultqvist
Johannes Ortner

Kiruna Geophysical Observatory,
Kiruna C., Sweden.
Dec. 18.

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Observations of Ionization induced by Artificial Earth Satellites

While monitoring a remote continuous-wave station at the Ohio State University Radio Observatory, it has been found that large enhancements of signals often occur at the times of near approach of an artificial Earth satellite. The enhancements are of such magnitude as to indicate extensive ionization with scattering cross-section several orders of magnitude greater than the physical cross-section of the satellite[1–3]. In these observations it has been convenient to monitor the National Bureau of Standards time service station *WWV* near Washington, D.C., at a distance of 330 miles from Columbus. This communication describes some additional observations of the phenomenon.

Between January 9 and February 7, 1959, the enhancements of signals of *WWV* on 20 Mc./s. were recorded on three or four passes of *Sputnik III* each night (between local midnight and 6 a.m.), while recordings of the satellite transmissions on 20·0045 Mc./s. were recorded to determine the time of near approach. The passes during this period were typically west to east with near approach over Hudson Bay (north of Columbus). A three-element beam antenna directed to the north was used for reception. The *WWV* records were divided into approximately 100-min. intervals centred on the near approach time of *Sputnik III*. All *WWV* enhancements of more than a constant minimum magnitude and more than 30 sec. in duration were taken as significant. Adding all such enhancements (about 200) for a total of 50 passes on 14 nights (3–4 passes per night) a histogram was obtained. Averages for each 5-min. period were taken and a smoothed curve drawn through these values. The result is shown in Fig. 1.

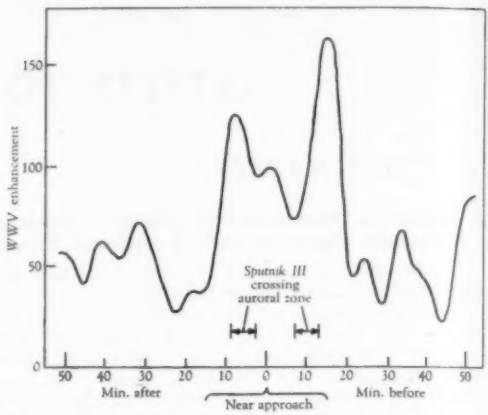

Fig. 1. Enhancements of *WWV* 20-Mc./s. signals as a function of time before and after the near approach of *Sputnik III* for 50 passes on 14 nights during January and February 1959

This graph gives the amount of *WWV* enhancement (number of enhancements times magnitude) to an arbitrary scale as a function of time before and after the near approach of *Sputnik III*. A peak occurs at the time of near approach, but larger peaks somewhat before and after. These three peaks, grouped around the near approach, are as much as 10 times greater than the standard deviation in the remainder of the graph, which makes it very unlikely that they are due to a random process. It is to be noted that the largest peaks are 15 min. before (precursor enhancement) and 8 min. after near approach (post-enhancement). It may be significant that these times correspond approximately to the times the satellite was, on the average, passing through the auroral zone (see Fig. 1). A possible hypothesis for explaining this effect is that the satellite encountered regions of high-particle density in the auroral zone, scattering material into ionized clouds or streams which travelled closer to Columbus with reflexions from these clouds causing many of the observed signal enhancements.

In order to obtain more data on the ionized clouds, a radar transmitter of about 600-W. peak power was installed at the prime focus of the Ohio State University 360-ft. radio telescope[4]. At the time, the parabola was completed but the flat reflector had not been assembled, so that the parabola was unobstructed to the north. Using frequencies between 17 and 19 Mc./s., a fan beam about 10° wide by 50° high was produced above the northern horizon, with a gain of nearly 20 decibels. The receiver was located about 20 miles south at Columbus. The receiver output was displayed on a *z*-type oscilloscope and was recorded on 35-mm. film moving 2 cm./min. With this arrangement a number of significant records were obtained. Fig. 2 shows one of these, obtained on February 20, 1959. Time increases to the left and range increases vertically downward. Between 11.13 and 11.15 p.m., *Sputnik III* was in the beam of the 360-ft. parabola travelling north-west to south-east at an average range of about 1,400 miles (height 860 miles). At 11.13 several traces appear on the film which radiate from the point *A*. At 11.13·8 two traces appear on the film which radiate from the point *B* and at 11.14·8 there are two traces radiating from point *C*. Points *A*, *B* and *C* are all close to the range of the satellite (dashed line) as calculated from ephemeris and other data with the satellite reaching its point of near approach north-east of Columbus about 11.17 p.m. at a range of about 1,200 miles. At point *C* there are two faint traces close to the range of the satellite. These may be due to reflexions from ionization at or close to the satellite.

It is postulated that at points *A*, *B* and *C* the satellite encountered regions of high density scattering a number of particle clouds. Clouds from the radiant points *B* and *C* approached within about 400 miles of Columbus. (Similar particle clouds may also account for the precursor enhancement peak in

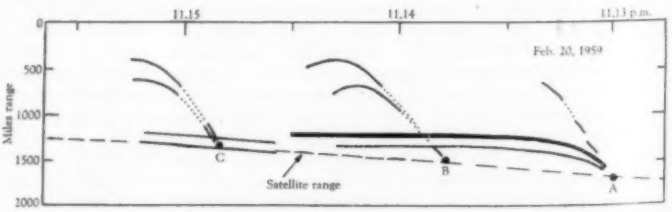

Fig. 2. Radar traces of moving ionized clouds which appear to originate at the range of an artificial Earth satellite (*Sputnik III*) as it passed through the beam of the 360-ft. parabola of the Ohio State University radio telescope

Fig. 1.) The two traces lasting about 90 sec. extending from the radiant point A start out with hyperbolic shape but flatten out to a constant range, suggesting that the particular clouds of particles producing them left an ionized wake nearly perpendicular to the radius vector from Columbus. From the slopes of the traces in Fig. 2, the velocities of the particle clouds radiating from A, B and C are between 30 and 150 km./s. These values are much in excess of the actual velocity of the satellite of about 7 km./s. Such velocities might be explained by collisions of the satellite with fast corpuscular streams. Assuming elastic collisions and an exactly head-on geometry of encounter, the resulting particle-velocity is equal to the velocity of the incident stream plus twice that of the satellite.

It should be mentioned in connexion with Fig. 2 that at about 11.13 p.m. the signals from the *Sputnik III* transmitter on 20·0045 Mc./s., which had been very strong, dropped to a very low level for about 1 min., after which their strength returned. The decrease in signal coincided with the beginning of the radar traces from the radiant point A, suggesting the possibility that the ionized particles encountered or produced at this time resulted in absorption or scattering of the signal from the transmitter on the satellite, while at the same time the particles acted as a reflecting or back-scattering medium for the radar signals.

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John D. Kraus
Robert C. Higgy
Donald J. Scheer
W. Reed Crone

Radio Observatory,
Ohio State University,
Columbus, Ohio.
Dec. 7.

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Temperatures within the Earth's Core

In a recent communication, Strong[1] gave the results of an experimental determination of the fusion curve of iron up to a pressure of 96,000 atmospheres. On the usual assumption that the Earth's core is composed of iron, he extrapolated his results to obtain a value of 2,610 ± 200° K. for the melting point in the core at the core–mantle boundary. His experimental results fitted Simon's[2] equation :

$$\frac{p}{a} = \left(\frac{T_m}{T_0}\right)^c - 1 \qquad (1)$$

with the constants $a = 75{,}000$ atmospheres and $c = 8$. In this equation p is the pressure in atmospheres, T_0 the melting temperature in degrees Kelvin at one atmosphere (that is, $T_0 = 1{,}805°$ K.), and T_m the melting temperature at the pressure p. Estimates of the melting point in the Earth's core obtained by Strong are appreciably lower than those obtained using Simon's values for the constants a and c, and only about one-half of those obtained using Gilvarry's[3] more recent values. Moreover, the gradient of the melting point curve is much lower than in these previous estimates. The value of 2,610° K. for the melting point at the core–mantle boundary is particularly interesting in view of Verhoogen's[4] conclusion that the temperature there is not likely to exceed 2,700° K. and his previous estimate[5] of 2,500° C. as the maximum, which was based on the hypothesis that the maximum observed surface temperature of lavas reflects the maximum temperature in the mantle. Since seismic evidence indicates that the outer core in any event is liquid, these two results of Strong and Verhoogen taken together fix the temperature T_1 at the core–mantle boundary rather precisely, namely :

$$2{,}610 < T_1 < 2{,}700 \qquad (2)$$

so that a value of 2,650° K. for T_1 seems reasonable.

The only estimate of the melting-point temperature in the mantle is that due to Uffen[6], who obtained a value of 5,000° K. at the core–mantle boundary. With the low melting-point temperatures in the core as indicated by Strong it is worth reconsidering the early history of the Earth's interior. In my[7] explanation of the solid inner core for an Earth cooling from a molten state, it is necessary to postulate a discontinuous decrease in the melting point at the core–mantle boundary, and a higher melting point at the centre of the Earth than at the bottom of the mantle. This last requirement is certainly not met now unless Uffen's figures can be considerably reduced. A completely molten Earth would thus solidify from the core boundary and progress outwards, leaving the entire core liquid. A solid inner core could arise, however, on the hypothesis of a cold origin for the Earth, in which case melting would progress inwards from the core–mantle boundary as the temperature of the interior increased. As shown below this seems very probable.

The flatness of the gradient of the melting point curve makes it instructive to re-estimate the adiabatic gradient in the core. Vallé's estimate[8], based on seismic data and solid state theory, was greater than Simon's value of the melting point gradient and is much greater than the new values of Strong. If this is true, it would be impossible for an Earth cooling from a molten state to leave a solid inner core and a liquid outer core. Far more important, convection currents in the core which are required by most modern theories of the Earth's magnetic field, could not occur. I have thus re-computed the adiabatic gradient in the outer core, on the basis of my temperature–pressure hypothesis[9]. On this theory, the adiabatic gradient depends on the temperature and is given by :

$$\frac{dT}{dp} = \frac{T}{\rho\, c_p\, (C + bp)} \qquad (3)$$

where ρ is the density, c_p the specific heat at constant pressure and C and b constants ($C = 2{\cdot}4 \times 10^{4}$° K., $b = 6{\cdot}2 \times 10^{-8}$° K. cm.²/dyne). Equation 3 can be integrated to give :

$$\log_e T = \int \frac{dp}{\rho\, c_p\, (C + bp)} \qquad (4)$$

Assuming c_p to be constant[9] ($1{\cdot}24 \times 10^{7}$ ergs/gm. ° K.), and the temperature T_1 at the core–mantle boundary to be 2,650° K. (cf. the inequality (2)), the adiabatic temperature can be estimated at any depth within the core, and, by differentiation, the

adiabatic temperature gradient. It is found that the melting-point gradient exceeds the adiabatic temperature gradient so that convection in the core would be possible. The adiabatic temperature at the boundary of the inner core estimated from equation (4) is 2,900° K. The melting point at this depth, calculated from equation (1), has exactly the same value. Although the agreement between these two estimates may well be fortuitous, it would appear that the inner core is solid.

J. A. Jacobs

Geophysics Laboratory,
University of British Columbia,
Vancouver, Canada.

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PHYSICS

Graphitization of Diamond

In an earlier article in *Nature*[1] I reported some results of a study of the high-temperature transformation of diamond to graphite. In this work diamond powder of 0–1μ particle size was used. Samples which had been heated in vacuum to various temperatures in the range 1,000–2,000° C. were obtained and these were investigated by microscopy and diffraction in a Siemens electron microscope (Elmiskop I). Specimens which had not been heated above about 1,200° C. gave diamond diffraction patterns only, those which had been heated to 2,000° C. gave graphite patterns only, and those which had been heated to about 1,600° C. gave both diamond and graphite patterns and also some extra spots not due to either diamond or graphite. It was suggested that these extra spots were due to some intermediate arrangement of carbon atoms, possibly a regular array of diamond-like and graphite-like regions produced during the graphitization. More recent work has shown that this interpretation of the results is not justified, and it is the purpose of the present communication to describe the diffraction patterns in more detail and to give such interpretation of them as at present seems reasonable.

The transmission electron diffraction patterns were complicated, and it was not possible to obtain great accuracy in the calculated Bragg spacings because of refraction effects and other distortions of the patterns. Except for two specimens the highest spacing found was between 3·0 and 3·1 Å. The two exceptions showed reflexions at spacings of 6·0 Å. for the one and 4·1 and 3·6 Å. for the other. Lower spacings were 2·8, 2·6, 2·5, 2·4, 2·2 Å., etc., but more accurate values were afterwards obtained from X-ray powder photographs (obtained by Dr. P. Gay, Department of Mineralogy, Cambridge). These gave the values 2·60, 2·55, 2·50, 2·36, 2·18, 1·99, 1·89, 1·535, 1·419, 1·310, 1·134, 1·086 Å.

It was also found (originally by A. I. Bailey and J. W. Menter in 1950, private communication) that, if large single-crystal diamonds which had been heated to red heat in a flame were examined by reflexion electron diffraction, both regular cross-grating spot patterns and continuous ring patterns could be obtained. The spot patterns gave spacings of 8·7, 6·2, 5·8, 4·4, 3·6, and 2·5 Å. The high-spacing part of the ring patterns was lost in the shadow of the specimen edge; but rings 3·1, 2·8, 2·63, 2·52, 2·35, 2·18 Å. and lower were seen. The spacings were the same as in the transmission patterns of graphitized diamond dust. These reflexions, with the exception of those at 5·8 and 2·55 Å., can all be interpreted in terms of a cubic structure of cell side 12·3 Å. Almost all the results can thus be accounted for, and it was thought at the time that the earlier communication[1] was written that an intermediate phase must have been formed since no likely impurity having a unit cell of these dimensions exists. Furthermore, the appearance of dark-field electron micrographs suggested that the material having this structure was distributed uniformly and homogeneously through the diamond sample[2].

However, the diamond samples which gave these diffraction patterns were found by spectrographic analysis (done by Messrs. Johnson Matthey, Ltd.) to contain about 1 per cent of silicon. When the experiments were afterwards repeated using a purer diamond sample (0·0002 per cent silicon), no extra reflexions were found in the transmission electron diffraction patterns, and only one very weak extra reflexion—at 1·89 Å.—in the X-ray powder photographs. It therefore seems that a silicon compound was responsible for nearly all the extra reflexions and it is almost certain that this was silicon carbide. With the exception of the line at 1·89 Å., all the extra lines in the X-ray powder photographs agreed in spacings and relative intensities with those in a comparison pattern from silicon carbide. The spacings from most of the extra spots in the transmission electron diffraction patterns agreed with the values for silicon carbide, but there were a few exceptions—weak reflexions at 6·0, 4·1, 3·6, 3·0, and 2·8 Å. These reflexions were presumably due to some other impurity; but this has not yet been identified.

The flame-heated diamonds also gave extra reflexions when examined by reflexion electron diffraction. The ring patterns gave the same spacings as were found for the graphitized diamond dust and presumably were caused by the presence of the same impurities. The cross-grating spot patterns gave larger spacings than were found from the other patterns. They may well be due to an impurity; but again this has not yet been identified. The fact that nearly all the lattice spacings fit a cubic lattice of cell side 12·3 Å. seems to be fortuitous, especially as most of the larger spacings were only observed with the one type of specimen.

It is concluded that the hypothesis of an intermediate structure of carbon atoms is not justified, and that the extra spots and rings in the diffraction patterns were probably caused by the presence of silicon carbide and other unidentified impurities. Silicon carbide has also been found to be a contaminant of heated diamond by Raal, whose results are published in the following communication. The two sets of results are in good agreement and show the formation of silicon carbide at similar temperatures; but the source of the silicon was different—in Raal's case it was the fire-clay boat used to contain the diamond, in mine an impurity in the diamond sample.

I wish to thank Dr. F. P. Bowden for his interest in this work, and Messrs. Industrial Distributors (1946), Ltd., for a grant to the Laboratory.

M. Seal

Research Laboratory for the Physics and Chemistry of Solids,
Department of Physics,
University of Cambridge.

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Alleged Formation of an Intermediate Diamond Structure on Heating Diamond

It was shown by Seal[1] that little change was produced by heating natural diamond powders to 1,200° C. in a vacuum (10^{-4} mm. mercury) for periods of 15–30 min. Heating the powders to 2,000° C. resulted in a complete transformation to graphite. For temperatures of about 1,500° C. both diamond and graphite could afterwards be detected, and, in addition, the electron diffraction patterns showed extra spots not due to either diamond or graphite. Seal advanced the theory that these extra reflexions were due to some intermediate arrangement of carbon atoms in diamond, possibly a regular array of diamond and graphite-like regions.

X-ray diffraction patterns of the samples gave extra lines, by far the strongest of which corresponded with a lattice spacing of 2·51 Å. Also, it was stated that spectrographic analyses of the diamond powders before and after heating showed a chief impurity of 1 per cent silicon (Seal, M., private communication).

In a current programme of investigation diamond powders have been heated to about 1,400° C. in an inert atmosphere for various lengths of time. X-ray as well as spectrographic analyses of the powders before and after heating have confirmed the observation of Seal; but, in the light of our experiments, the existence of an intermediate atom structure in diamond after heat treatment can be discounted, and an obvious explanation for the extra X-ray reflexions and the increased silicon content of the heated diamond powder is given.

On measuring the extra lines of the X-ray pattern of the heated diamond powders the strongest reflexion was found indeed to correspond with a lattice spacing of 2·51 Å. In addition, two reasonably strong reflexions for spacings of 1·54 Å. and 1·31 Å. were found. Reference to the ASTM X-ray data card system identified these extra reflexions as being due, almost certainly, to silicon carbide.

This result is significant in the light of the fact that a chief impurity of 1 per cent of silicon is quoted by Seal for the heated diamond powders, whereas a silicon content of more than 0·1 per cent is seldom found for natural unheated diamond[2].

According to Tammann[3] the reaction between silicon and carbon to form silicon carbide starts at 1,340° C.

It appears, therefore, that the extra X-ray reflexions as well as the high silicon impurity content of the heated diamond powder are attributable to the formation of some silicon carbide as a result of chemical reaction between the diamond powder and silicon at an elevated temperature. In Seal's experiments the heating was done in crucibles of pure graphite; but the silicon might have been present initially as a contaminant or might have originated from his furnace. In our experiments, boats of fire-clay, which is essentially an aluminium silicate, were used for the heating, and it is very likely that at 1,400° C. or higher they react either with the pure diamond or a graphitized layer on the surface of the diamond to give silicon carbide.

In order to verify this a diamond powder was heated at 1,400° C. in a platinum spoon for several hours and analysed by means of X-rays as well as spectrographically. Extra X-ray reflexions were completely absent, and as can be seen from Table 1, the silicon content is not more than normally found in diamond powder.

Table 1

Diamond powder	Silicon content (per-centage by weight)	Extra X-ray reflexions
Unheated	0·01	No
Heated to 1,400° C. in fire-clay boat	1·00	Yes
Heated to 1,400° C. in platinum spoon	0·04	No
Heated to 1,400° C. in fire-clay boat and treated in hydrofluoric acid	1·00	Yes
Heated to 1,400° C. in fire-clay boat and subjected to sodium-hydroxide–sodium nitrite fusion	0·01	No

A further test was done to substantiate whether, in fact, silicon carbide is formed and whether the increased silicon content of the powder heated in the fire-clay boat is not just a result of contamination from the latter.

The heated powder was treated for several hours in hot hydrofluoric acid which digests silicon in any form except as the carbide. The extra X-ray reflexions and high silicon content of the powder remained unaltered as is evident from Table 1. On subjecting this powder to a sodium hydroxide–sodium nitrite fusion which is capable of removing silicon carbide, the silicon content, as depicted in Table 1, becomes normal again and the extra X-ray reflexions are absent.

From the above considerations it is evident that the extra X-ray and electron diffraction spots observed by Seal in heat-treated diamond powders are due to the existence of easily detectable amounts of silicon carbide as a result of chemical reaction at elevated temperatures.

I wish to thank Drs. H. J. Nel and A. R. Roy for their interest and helpful discussions and Messrs. Industrial Distributors (1946), Ltd., for permission to publish this communication.

F. A. Raal

Diamond Research Laboratory,
Johannesburg, South Africa.

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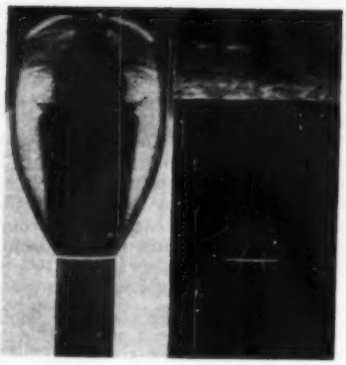
Emission and Absorption of Light behind the Detonation Front

When a high explosive detonates, the measurement of the true emission of light from the detonation gases is of great interest. In air, however, the expanding detonation gas is enveloped in a compressed layer of the displaced air, which acquires a high temperature (*c.* 8,000° K. at atmospheric pressure) and emits an intense light. An easy way of eliminating the luminous layer of air is to immerse the charge in water. Charges of *TNT* and phlegmatized *PETN*, for example, then show a narrow luminous zone



(*a*) (*b*)

Fig. 1

behind the detonation front. Fig. 1 shows instantaneous pictures (exposure time, 0·03 μsec.) of detonating cylinders of phlegmatized *PETN* of 1·55 gm./cm.³ density and 25-mm. diameter, first with (1*a*) then without (1*b*) background light of a detonated *TNT*-ball. (In Fig. 1*b* and Fig. 2 the upper end of the charge was above the surface.)

Fig. 1*a* shows that the expanding detonation gas is non-transparent to the background light. This implies that absorption of light is important in determining the light observed. In the case of phlegmatized *PETN* (10 per cent wax), which is very deficient in oxygen, this may be due to particles of carbon forming a light-absorbing screen on the cooled surface layer of the detonation gas. C. H. Johansson and L. Sternhoff[1] have recently shown that in *TNT*, even the narrow band of light disappears if the charge is exposed to the pressure wave from a neighbouring charge which has detonated somewhat sooner. In view of these results, the visible luminous zone cannot be assumed to correspond to the reaction zone.

In order to eliminate the absorption of light by carbon particles, similar tests were made with blasting gelatine freed from air bubbles. Instantaneous photographs show that light is emitted from a greater area with gradually decreasing intensity backwards. The diameter of the charge is, however, possibly too small for black-body emission.

Fig. 2

To get the intense light emission of *PETN* without absorption at the surface, we have made tests with charges with a varnished core of phlegmatized *PETN* and a surface layer of blasting gelatine intended to prevent the cooling off at the surface of the detonation gases of *PETN*. The coating of varnish prevented the dissolution of the *PETN* by the blasting gelatine. Fig. 2 shows an instantaneous photograph of a detonating charge of a 25-mm. *PETN* cylinder coated with a 1-mm. layer of blasting gelatine freed from air bubbles. In this case the light is emitted over a wide area behind the front and corresponds probably much better to the temperature distribution in the expanding detonation gas of *PETN* than the narrow luminous zone in Fig. 1.

This investigation has been carried out with support of the Swedish Detonation Research Foundation.

C. H. Johansson
T. Sjölin

Physical Research Department,
Nitroglycerin AB.,
Stockholm.

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Effect of Corona on Travelling Waves

A new phenomenon of impulse corona has been observed with voltage waves of relatively long wave-fronts on a 33-kV. overhead line. The wave-shape at the sending end is 5·7/19·3 μsec. nominal. The accompanying oscillograms show typical waves recorded at a point 2·08 miles away. As the voltage exceeds the corona threshold, the wave-front shows the well-known phenomenon that part of it is sheared off due to corona losses. However, the voltage clearly recovers before its peak value is reached whereas previous observations made with wave-fronts of 1·5 μsec. or less show that this shearing effect extends to the wave tail. After the tests on which this communication is based had been completed, it was found that this recovery of voltage had also been observed by Mita and others[1] on a model line with waves of 2·2 and 4·2 μsec. fronts. As can be seen from the oscillograms, the rate of recovery is much faster for a negative wave than for a positive wave.

This phenomenon suggests that the initial intense ionization is halted, completely or almost completely, by the space charge of its own creation. This view is supported by the cessation of the high-frequency oscillation on the positive wave just before the voltage recovers. This high-frequency oscillation had also been observed to be associated with positive corona during earlier tests on 33-kV. and 132-kV. lines[2]. The fact that this recovery of voltage was not previously observed is probably due to the short front-durations of the impulse waves employed so that only the effect of the initial stage of the corona process became apparent.

Clearly, the distortion of the wave front due to corona cannot be considered to be independent of the front-duration as has been assumed in existing theory[2,3]. Either the theory has to be modified or a new approach adopted.

Several authors have measured the relation between impulse voltage $e(t)$ and corona charge $q(t)$. The results obtained by Aso[4] on a thin wire (0·6 mm. diam.) inside a concentric cylinder (10 cm. inside dia.) show that when the voltage exceeds the corona threshold dq/de increases rapidly to a fairly constant value and then decreases to a small value. It can be

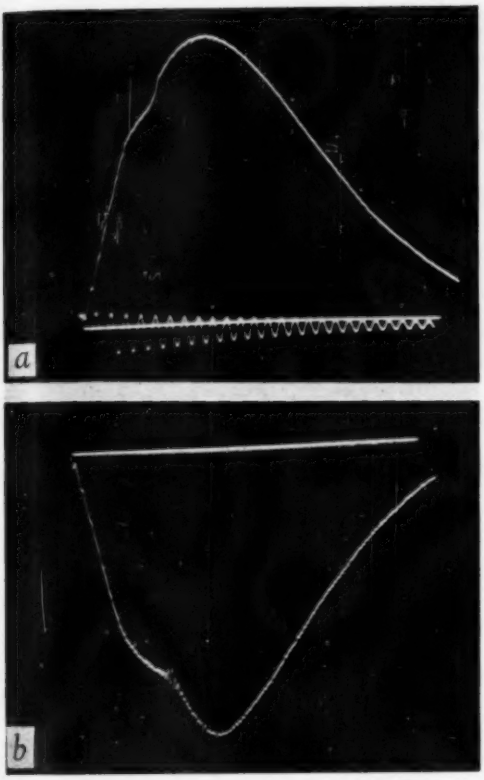

Fig. 1. *a*, Negative wave (timing oscillation, 1 Mc./s.); *b*, positive wave

shown mathematically that if the $q \sim e$ curve for the test line shows this variation in dq/de then the voltage will recover as observed.

A full report will appear elsewhere. Acknowledgment is due to the Director of the Electrical Research Association for permission to publish this communication.

M. OUYANG

Electrical Research Association Laboratory,
Leatherhead, Surrey.
Jan. 15.

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RADIATION CHEMISTRY

Effect of Ionizing Radiation upon Xanthosine in Dilute Aqueous Solution

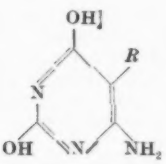
IT was reported earlier[1] that irradiation of an aqueous solution of guanosine with 15 MeV. electrons opened the imidazole ring to form 2 : 4-diamino-5-formamido-6-hydroxypyrimidine riboside. This communication reports that a similar ring-opening occurs when an aqueous solution of xanthosine is irradiated.

An aqueous solution of xanthosine, concentration 10^{-3} *M*, and containing 10^{-3} *M* neutral phosphate buffer, was irradiated with 15 MeV. electrons to a dose of 8×10^5 rads. The irradiated solution and the control solution were analysed by paper chromatography, using an 85 per cent saturated aqueous solution of ammonium bicarbonate as a solvent[2] (solvent 1). While the control solution gave a single ultra-violet absorbing band, the irradiated solution was resolved into four bands (Table 1) which were identified by their R_F's in solvents 1, 2[3] and 3[4], and the ultra-violet spectra of their eluates.

Table 1. R_F VALUES OF ULTRA-VIOLET ABSORBING COMPOUNDS OBTAINED FROM AN IRRADIATED AQUEOUS SOLUTION OF XANTHOSINE

Band	Solvent 1 (ref. 2)	Solvent 2 (ref. 3)	Solvent 3 (ref. 4)	Identity
1	0·46	0·31	0·67	xanthine
2	0·64	0·20	0·90	xanthosine
3	0·73	0·18	0·81	I
4	0·85	0·09	0·94	I-riboside

Band 1 was identified as xanthine, and is assumed to be liberated by hydrolysis following radical attack upon the ribose moiety[5]. Band 2 is unchanged solute. The spectra of bands 3 and 4 were similar with a single absorption maximum, the maximum of band 4 being at a longer wave-length (270 mμ) than that of band 3 (262 mμ). Analogy with results obtained for guanosine[1] suggested that band 3 is 2 : 6-dihydroxy-4-amino-5-formamido-pyrimidine (I) and band 4 its riboside.



I. (R = NHCHO)

II. (R = NH_2)

The identity of band 3 was confirmed by comparing its R_F's and ultra-violet spectra with those of an authentic specimen of I, kindly supplied by Mr. G. M. Timmis. Their R_F's in solvents 1, 2 and 3 (Table 1), and their ultra-violet spectra at *p*H 7 and 12 were identical (Fig. 1). At *p*H 1 and room temperature the ultra-violet absorption decreased slowly. In view of the well-known acid lability of formyl groups, it seemed possible that I was slowly deformylated in acid to 2,6-dihydroxy-4,5-diaminopyrimidine (II). This was essentially confirmed when, after leaving I in 0·1 *N* hydrochloric acid for 48 hr., the resulting spectra were found to be identical with those published for II [6]. Accordingly, an acid spectrum of I was obtained by dissolving it in 98 per cent formic acid (see Fig. 1), which gave no spectral change over a period of 20 hr.

Band 4 was identified as the riboside of I by identifying the sugar and ultra-violet absorbing compounds released during acid hydrolysis. Band 4 was separated from free ribose by running it in solvent 2, eluted and hydrolysed in 85 per cent formic acid at 100° C. for 5 min. When the hydrolysate was run in solvent 2 an ultra-violet absorbing product was detected with R_F's and ultra-violet spectra identical with those for I. A sugar spot, with an R_F identical with that for ribose, was detected by spraying the paper with aniline-hydrogen-phthalate[3]. Xanthosine is readily hydrolysed in acid[5]. Under the conditions

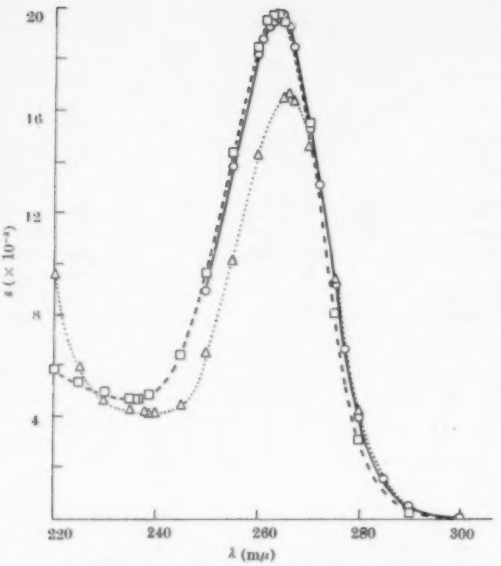


Fig. 1. Ultra-violet absorption spectra of 2 : 6-dihydroxy-4-amino-5-formamido-pyrimidine (I). ○—○, in 98 per cent formic acid ; □ - - - - □, at *p*H 7 ; △ · · · · △ at *p*H 12

described above xanthosine and I-riboside (band 4) were completely hydrolysed, while under milder conditions, namely, 85 per cent formic acid at room temperature, half the starting material was hydrolysed in 22 hr. and no difference in the rates of hydrolysis of xanthosine and I-riboside was observed.

It is now established that the imidazole ring is opened when aqueous solutions of guanosine and xanthosine are irradiated. Results obtained with adenosine and inosine, to be published shortly, show that here too the imidazole ring is opened ; this seems to be a general reaction for purine nucleosides. When an electrophilic reagent approaches the purine ring C–8 appears to have the highest electron density[10]. Assuming that the chemical action of ionizing radiation is by OH radicals[11], attack at C–8 would be consistent with the electron configuration of the purine ring, mentioned above. Xanthosine is changed at a rate ($=G$) of 1·2 molecules per 100 eV. absorbed energy, which is low compared with the rate of formation of OH radicals ($G=2{\cdot}8$)[12]. Presumably the reaction is complicated by unknown factors, for example, a back-reaction, upon which the present results shed no light.

I wish to thank Prof. J. Rotblat, St. Bartholomew's Hospital Medical School, for his kind permission to use the linear accelerator. The Medical Research Council is thanked for its financial support when this work was begun at the Barnato-Joel Laboratories, Middlesex Hospital, London. Identification of products was completed at the Chester Beatty Research Institute, Institute of Cancer Research, with the financial support of the William Shepherd Fund, and by grants to the Chester Beatty Research Institute (Institute of Cancer Research : Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

G. Hems*
Chester Beatty Research Institute,
London, S.W.3,
and
Barnato-Joel Laboratories, Middlesex Hospital,
London, W.1.

* Present address : Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3.

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CHEMISTRY

Simple Non-gassing Electrodes for Use in Electrophoresis

The electrodes conventionally used in electrophoresis are noble metal (usually platinum), metal/metal ion (for example, Cu/Cu^{2+}) and metal/insoluble metal salt (for example, $Ag/AgCl/Cl^-$). With the last two types the apparatus must be provided with convection barriers to prevent metal ions in the electrode compartments from contaminating the experimental material. Platinum electrodes, even if platinized, permit only a limited current density (less than 2 m.amp./cm.^{2}) without giving an evolution of gas bubbles which disturbs the observations in closed electrophoresis systems such as are used in the moving boundary and microscopic methods. We wish to describe here the general conditions under which palladium–hydrogen electrodes can be used to provide adequate current densities for electrophoresis without gassing or danger of contamination from heavy metals.

Almost a century ago Graham found that palladium metal would dissolve considerable amounts of hydrogen when used as the cathode in an electrolytic cell[1]. Present evidence indicates that the following electrolytic reactions take place at palladium electrodes[2] :

$$xH_3O^+ + Pd + xe^- = PdH_x + xH_2O \text{ (at low } p\text{H)}$$
$$xH_2O + Pd + xe^- = PdH_x + xOH^- \text{ (at high } p\text{H)}$$

The PdH_x formed at the cathode is not a definite chemical compound such as a hydride, but more nearly an alloy of palladium with hydrogen[3].

So long as the cathodic formation of PdH_x is fast compared with the combination reactions of atomic hydrogen on the surface of the electrode, no molecular hydrogen will be evolved until the solubility of hydrogen in palladium is exceeded at a hydrogen/palladium atomic ratio of about 0·9 (ref. 4). At the anode, oxygen or other gases will not be evolved so long as the electrode has a store of atomic hydrogen and the current density is not excessive. Obviously the anode must be cathodically charged with hydrogen before use.

While similar reactions may occur at platinized platinum electrodes, the situation is considerably altered by the fact that the total amount of hydrogen stored is limited to surface adsorption. The advantage of palladium as a current-carrying electrode lies in its capacity to absorb considerable amounts of hydrogen.

Three possible ways of using palladium electrodes are indicated schematically in Fig. 1,*a*, *b*, *c*. In the simplest system, Fig. 1,*a*, the electrodes are immersed in the solution, filling the electrode chamber. Here the anode must be precharged with an amount of hydrogen sufficient for the electrophoretic run. Relatively small electrodes can store ample amounts of hydrogen for many purposes; for example, a palladium anode weighing 1 gm. will, if precharged to a hydrogen/palladium atomic ratio of 0·5, deliver a current of 10 m.amp. for more than 12 hr. before the hydrogen is exhausted.

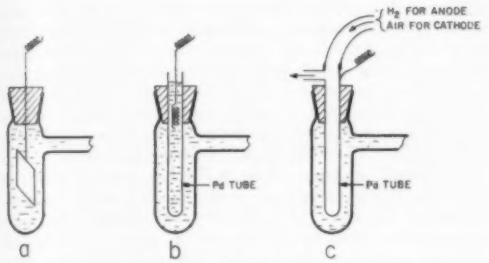

Fig. 1

We have used successfully electrodes of this type with conventional flat microelectrophoresis cells over a period of a year in studies on erythrocytes and bacteria. Current densities in excess of 10 m.amp./cm.^{2} of electrode area could ordinarily be used without gas evolution. Thus quite small electrodes are suitable even for studies at high ionic strengths.

In Fig. 1,*b*, the palladium tube serves as a bielectrode separating the solution leading to the electrophoresis cell and the solution surrounding the primary electrode (for example, Ag/AgCl). Here gas evolution resulting from exhaustion or saturation of atomic hydrogen at the electrodes would not occur regardless of the duration of the current since equivalent amounts of hydrogen are released and taken up by opposite faces of each bielectrode. The choice of the half-cell to be used inside the palladium anode tube should be made with care since the presence of soluble oxidants such as oxygen would result in the consumption of atomic hydrogen present on the palladium surface before it could enter the bulk of the metal. Hence the supply of hydrogen in the metal would eventually be depleted and gas bubbles might form at the exterior surface of the tube.

In the cell of Fig. 1,*c*, the interior of the palladium tube anode is continuously flushed with hydrogen gas and the cathode is open to the air. On passage of current through the system the hydrogen dissolved in the anode from the gas phase migrates through the wall of the palladium tube and reacts at the solution interface as indicated in the equations given above. At the cathode, atomic hydrogen derived from the reduction of hydronium ions in the solution migrates through the wall of the tube and reacts with atmospheric oxygen to form water. With these electrodes, current could obviously be passed in one direction indefinitely with no danger of gas formation at either electrode. This system would not be convenient, however, where frequent reversal in current direction is necessary (as in microelectrophoresis) since the hydrogen and air connexions would have to be exchanged with each reversal. Electrodes of this type have been used successfully in an electrochemical cell[6].

A few precautions in the use of palladium electrodes are worth mentioning. A palladium anode immersed in a halide solution will react to give a soluble palladium halide salt if its store of hydrogen is exhausted. Charging a palladium electrode beyond a hydrogen/palladium ratio of about 0·6 will lead to an increase in volume of about 10 per cent[7]. Repeated cycles of charging beyond this ratio followed by complete discharge may cause the metal to crack. By restricting the maximum amount of hydrogen dissolved to a ratio of about 0·5 the electrodes may be expected to have an indefinite life.

REX NEIHOF
SIGMUND SCHULDINER
U.S. Naval Research Laboratory,
Washington 25, D.C.

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Separation of Uranium by Reversed Phase-Partition Chromatography

IN almost all analytical methods for the determination of uranium, it is necessary first to separate the uranium from other substances.

A convenient and selective method of achieving this separation is to extract the uranium from a nitric acid solution into a solution of tributyl phosphate in kerosene, the latter serving to facilitate separation of the two phases. Usually two extractions are required, one to transfer uranium to the organic phase and one to return it to an aqueous phase for determination.

The use of tributyl phosphate on a partition chromatographic column would reduce the hazard of manipulating radioactive material, improve the partition of uranium into the organic phase, and have the effect of a large number of manual solvent extractions. Such a column has been successfully used in these laboratories for the past eleven months (internal publication and patent application).

The stationary phase is prepared from purified tributyl phosphate. A mixture of 100 ml. of commercial tributyl phosphate and 150 ml. of 0·5 *M* sodium hydroxide is steam distilled until no odour of butyl alcohol is present in the distillate. After cooling, the residual tributyl phosphate is separated from the alkali and washed with 100 ml. of distilled water, followed by three 50-ml. portions of 3 *M* nitric acid. For the solid support, 'Low density, *Kel-F* 300 moulding powder' (Minnesota Mining and Manufacturing Co.) is ground, using solid carbon dioxide to cool the material, and the powder passing 100 B.S.S. and retained by 200 B.S.S. is collected. The column is prepared by adding 3 ml. of purified tributyl phosphate to 3 gm. of the '*Kel-F*' powder, mixing with a glass rod, and loading as a slurry in 5·5 *M*

nitric acid into a burette 7 mm. in diameter containing a loose plug of cotton-wool above the tap. The slurry is agitated with a glass rod to release air bubbles, and 5·5 *M* nitric acid is run through the column to consolidate the contents. A loose plug of cotton-wool is placed over the material to prevent disturbance of the surface.

The sample solution, 5–10 ml., containing not more than 50 mgm. of uranium in 5·5 *M* nitric acid, is poured on to the column. The uranium is retained as a clearly defined yellow band at the top of the column and impurities are eluted by washing with three 10-ml. portions of 5·5 *M* nitric acid at a rate of flow of 1–2 ml. per min. The uranium is then eluted with 35 ml. of distilled water at the same rate and determined by a suitable method.

The column is reactivated by washing with 20 ml. of 5·5 *M* nitric acid. The tributyl phosphate is slowly eluted, but one column packing can be used for more than fifty determinations, and the *Kel-F* from exhausted columns may be used as support material after washing with carbon tetrachloride, and drying.

Most impurities are eluted rapidly, the peak of the elution being reached when 10–12 ml. of eluent have passed through the column. The peak for the elution of uranium with nitric acid is not reached until 200 ml. of eluent have passed through and there is a clear volume of about 40 ml. between the two peaks. In order to save time, the eluent is changed to water after the impurities have been eluted.

Uranium has been separated effectively from more than ten times its weight of chromium, copper, aluminium, nickel, vanadium, calcium, iron, molybdenum, tungsten, sodium, potassium, fluoride, phosphate, sulphate, chloride and from concentrated solutions of citric acid which complex uranium strongly. The method is applicable from the microgram- to the gram-level by proportional adjustment of the size of column. Recoveries at the level of 5 mgm. of uranium have been in excess of 99 per cent, which has been adequate for the purpose in hand. We are confident, however, that a quantitative recovery is possible, and the method is being further investigated. A full report of the work will be published.

The tributyl phosphate/nitric acid column has been used to separate uranium from some of its daughter products.

Thorium, plutonium IV and cerium IV are retained on the column after washing with nitric acid, but cerium can be separated after reduction by sulphur dioxide to cerium III.

By modifying the technique so as to use hydrochloric acid in place of nitric acid throughout, it has proved possible to separate plutonium from uranium. The plutonium must be reduced to the trivalent form before loading on to the column. The uranium is retained on the column.

We are grateful to the managing director of the Production Group of the United Kingdom Atomic Energy Authority for permission to publish this communication.

A. G. Hamlin
B. J. Roberts

United Kingdom Atomic Energy Authority
(P.G.),
Chemical Services Department,
Capenhurst Works, Nr. Chester.
Dec. 29.

Evidence for the Existence of a Potassium Rhenium Hydride and its Bearing on the Nature of the (−1)-Oxidation State of Rhenium

This is a preliminary report of some observations we have made on the product of the reduction of potassium perrhenate with potassium metal in ethylenediamine–water solution. According to Bravo, Griswold and Kleinberg[1], and also Floss and Grosse[2], rhenium is reduced to the (−1)-oxidation state in this reaction, and the solid rhenide $KRe.4H_2O$ may be isolated from the products.

The reduction procedure and method of separating the unreacted potassium perrhenate ($KReO_4$) and the bulk of the potassium hydroxide from the product were as described by Bravo, Griswold and Kleinberg[1]. The grey-brown solid obtained in this manner was purified by dissolving it in an alkaline 10 : 2 methanol–water solution, centrifuging to settle out the brown-black solid matter (rhenium metal or a rhenium oxide), and then slowly adding the clear supernatant to a large excess of oxygen-free, anhydrous *iso*propanol. The result is a white precipitate which was successively extracted three times with *iso*propanol and then three times with anhydrous ethyl ether; after which it was pumped down for 2 hr. at 90° C. This product is stable in an inert atmosphere.

On treating the white solid or its solution in aqueous alkali (which is quite stable) with various oxidizing agents (hypochlorite, thallous ion, permanganate) gas evolution takes place. The gas evolved when a solution of the solid in 5 *M* potassium hydroxide reacted with 0·1 *M* potassium permanganate was shown, by mass-spectrometric analysis, to be pure hydrogen. The volume of hydrogen evolved on treating about 50-mgm. samples of the solid with 25 ml. of a solution 4·5 *N* in alkali and 1 *N* in permanganate is about 10 ml. at room temperature and atmospheric pressure. We find, as the mean of three measurements:

gram atoms H evolved/gram atoms Re = 5·54 ± 0·06

In acid solution the solid decomposes with the evolution of hydrogen and the precipitation of rhenium metal. Treatment of about 55-mgm. samples with excess of dilute sulphuric acid yields about 20 ml. of hydrogen at room temperature and atmospheric pressure. As the mean of three measurements we find:

gram atoms H evolved/gram atoms Re = 10·05 ± 0·05

The above results indicate that the white solid contains a hydride of rhenium. To confirm this, about 40 mgm. of the solid were dissolved in about 0·2 ml. of de-aerated 50 per cent potassium hydroxide solution, and the proton resonance spectrum was obtained with a high-resolution nuclear magnetic resonance spectrometer operating at a frequency of 60 Mc./s. The spectrum consists of two peaks separated by 14·7 parts per million. The low-field peak is very large and is due to the water–hydroxyl ion protons. The high-field peak is much smaller and can only be attributed to protons of a hydridic nature (H^-). The shift of the hydride peak with respect to the proton resonance of pure water is + 13·3 p.p.m. Wilkinson and co-workers have independently obtained a similar spectrum for the solution resulting from the reaction of aqueous potassium perrhenate with sodium amalgam (Wilkinson, G., personal communication). In addition, we have observed the proton resonance spectrum of the

solid dissolved in deuterium oxide (heavy water) containing about 35 per cent sodium deuteroxide (NaOD) (no proton resonance could be observed on the solvent). Again the spectrum consists of only two peaks separated by 14·7 p.p.m. This shows that the solid contains H_2O or OH^- groups as well as hydridic protons. The fact that only two peaks occur indicates that alcohol and ethylenediamine are absent, and this is confirmed by chemical tests. The ratio of the intensity of H^- peak/intensity of $H_2O - OH^-$ peak $\simeq 2$. Hence the ratio H^-/H^+ in H_2O or OH^- $\simeq 2$; but it must be noted that the sample used in the deuterium oxide experiment had been standing under vacuum for about one month and may have been partially dehydrated.

These observations demonstrate the existence of a soluble rhenium hydride. This is the first example of a soluble transition metal hydride which does not also contain complexing groups such as $C_5H_5^-$, CO or CN^- attached to the metal ion (bis*cyclo*pentadienylrhenium hydride and rhenium carbonyl hydrides are known[3]). We believe that it is now no longer necessary to postulate the existence of the rhenide ion, Re^-, or an aquo complex of this ion, since all the properties of the apparent (−1)-oxidation state of rhenium[1,2,4] may be explained as being due to a hydride of a positive oxidation state of rhenium. Work on this problem is continuing, and it appears possible that more than one rhenium hydride may exist. Further details will be reported at a later date.

This work was supported in part by the U.S. Atomic Energy Commission.

Note added in proof. Two measurements of the area beneath the high-field proton-resonance peak in deuterated basic aqueous solvent gave 7·3 and 6·5 for the ratio of hydridic hydrogen atoms to rhenium atoms in the compound. The measurement, expected to be reliable to about 20 per cent, was calibrated by the addition of a known quantity of normal-isotopic water to the deuterated solvent.

A. P. Ginsberg
J. M. Miller
J. R. Cavanaugh
B. P. Dailey

Department of Chemistry,
Columbia University,
New York 27.

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Formation of Volatile Compounds on Gas–Liquid Chromatography Columns

The communication by Weurman and Dhont[1] reporting the formation of volatile esters on gas–liquid chromatography columns using polyethylene glycol 600 prompts us to report our own findings with a similar stationary liquid phase.

When first preparing a column filling with polyethylene glycol 400, it was distributed on 'Celite 545' in a mixed solvent of acetone and diethyl ether which was afterwards removed by heating in an oven at 100° C. for 3 hr. During this period a strong smell of formaldehyde was noticed but this later disappeared, and the column was therefore packed and brought into use at 60° C. with nitrogen as carrier gas. The copper connexions of the thermistor detector became corroded and the base line irregular. The stock of polyethylene glycol 400 was afterwards tested and found to contain formaldehyde (chromotropic acid test) and formic acid (paper chromatogram by Dr. G. C. Whiting).

Since this time we have pre-treated the polyethylene glycol 400 as suggested by Adlard[2], namely, by heating to 100° C., but under a higher vacuum (0·01 mm. of mercury), for 16 hr. Satisfactory results have since been obtained, chromatograms of alcohols showing no abnormalities. It thus seems that polyethylene glycols as supplied by the manufacturers contain volatile impurities which should be removed before use as stationary phases. Our findings offer an explanation of the source of formic acid causing the esterification observed by Weurman and Dhont.

Margaret E. Kieser
D. J. Sissons

Long Ashton Research Station, Bristol.

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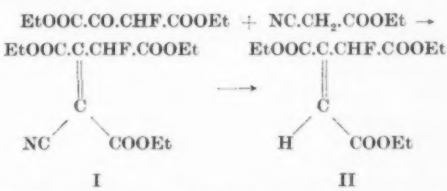
Triethyl α-Fluoroaconitate

The current theory on the mode of action of fluoroacetate[1] implies the lethal synthesis of fluorocitrate which poisons the enzyme aconitase, thus preventing the catabolism of citrate to aconitate; it seemed of interest to prepare and study α-fluoroaconitic acid, HOOC.CHF.C(COOH)=CH.COOH. Triethyl fluorocitrate, which would appear to be a suitable starting material, has been prepared in a facile reaction, namely, by the Reformatzky reaction between diethyl oxalofluoroacetate and ethyl bromoacetate[2], but our attempts to dehydrate this compound failed to give a defined product.

Better results were obtained by the condensation of diethyl oxalofluoroacetate with ethyl cyanoacetate in the presence of piperidinium acetate. The product so obtained (I) gave, upon treatment with alcoholic sulphuric acid, directly the desired triethyl α-fluoroaconitate (II).



I II

We have not succeeded in hydrolysing (II) without far-reaching degradation of the molecule. However, the biological properties of (II) as such will be of interest, as the complications inherent in the possible stereochemical forms of fluorocitrate[3] are not to be feared in (II).

Triethyl γ-cyano-α-fluoroaconitate (*I*). A mixture of 21 gm. of diethyl oxalofluoroacetate[4], 14 gm. of ethyl cyanoacetate, 0·5 ml. of acetic acid and 0·1 ml. of piperidine was heated for 20 hr. at 100° in a vacuum of 35 mm. After 6 and again after 13 hr. of this

period, 0·05 ml. of piperidine and 0·25 ml. of acetic acid were added. The product was subjected directly to distillation under 0·05 mm. pressure. The ester (I) boiled at 160–162° and formed a colourless oil; yield, 12 gm. (40 per cent).

Analysis: Calculated for $C_{13}H_{16}NO_6F$: C, 51·8; H, 5·3; N, 4·7; F, 6·3.

Found: C, 51·5; H, 5·6; N, 4·3; F, 5·9.

Triethyl α-fluoroaconitate (II). A mixture of 5 gm. of concentrated sulphuric acid and 1 gm. of water was added to a solution of 12 gm. of (I) in 20 ml. of anhydrous alcohol, refluxed for 5 hr. and poured into cold 10 per cent sodium hydrogen carbonate solution. Extraction with ether, drying and distillation gave 5 gm. (45 per cent) of triethyl α-fluoroaconitate (II), b.p. 115–117° (0·1 mm.).

Analysis: Calculated for $C_{12}H_{17}FO_6$: C, 52·2; H, 6·2; F, 6·9.

Found: C, 52·4; H, 6·3; F, 6·5.

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Ernst D. Bergmann
I. Shahak

Department of Organic Chemistry,
Hebrew University,
Jerusalem.

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Colorimetric Determination of Amino-acid Concentration

A versatile method of amino-acid determination is that in which amino-acid solutions are incubated with cupric orthophosphate in suspension. The resulting solution contains copper in proportion to the total molarity of amino-acids and the copper is determined by any suitable method. The methods used have included iodometric titration[1] and the use of cupric phosphate labelled with copper-64 followed by the determination of the activity in the supernatant[2]. The latter method is of particular use for the continuous determination of amino-acids in chromatographic eluates.

I have determined the copper colorimetrically using sodium diethyl dithio carbamate as the copper reagent. The colour produced is stable and has a maximum absorption at 440μ. The stages in the procedure are as follows.

(1) 0·1 ml. of the sample, 3·0 ml. of cupric orthophosphate suspension (1 per cent aq.), 2·0 ml. of B.D.H. Universal buffer solution, *p*H 7·0[3] are incubated overnight in a centrifuge tube. A reagent blank is used with distilled water replacing the sample. Using finely divided cupric orthophosphate (B.D.H.) shaking was found to be unnecessary even when using conical centrifuge tubes, the recovery figures being identical in procedures with and without shaking. The tubes are centrifuged to sediment the cupric orthophosphate and a part of the supernatant withdrawn for colour development.

(2) 0·1 ml. of the supernatant from (1), 3·0 ml. of B.D.H. Universal buffer *p*H 7·0, 0·1 ml. of sodium diethyl dithio carbamate 1·0 per cent aq., are mixed at room temperature. The optical density at 440μ does not increase after 15 min. from mixing, and is read at any convenient time, being stable for at least 24 hr. A 'Unicam' *S.P.* 500 or similar instrument is used with a violet filter. If the colour is too dense or if precipitation occurs, the supernatant from stage (1) is diluted and stage (2) repeated. The results obtained show that, due to the large excess of cupric orthophosphate in stage (1), the relationship [copper]/[amino-acid] is linear over a wide range, so that dilution is permissible with no inherent inaccuracy. The 'recovery' of added amino-acids was 100 per cent ± 2 per cent over a concentration range of 50–1,000 μgm./ml. α-alanine. This method has proved to be useful in enzyme kinetic work.

Nielsen[4] describes the estimation of proteins by a related method, involving the removal of amino-acids before reaction of the proteins and trichloracetic acid-insoluble peptides with cupric sulphate. Excess copper is removed by precipitation as cupric orthophosphate and the copper in solution as a peptide complex is estimated using diethyl dithio carbamic acid. In the method here described, peptides and proteins are found not to give any appreciable copper uptake from cupric orthophosphate, the results for amino-acid concentration obtained from solutions containing amino-acids, peptides and proteins being within 2 per cent of those obtained by formol titration and by the Folin–Ciocalteau method[5].

W. A. Vincent

Department of Biological Sciences,
Institute of Technology,
Bradford, 7.

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BIOCHEMISTRY

Volatile Amines in Brain

We have reported previously the isolation and identification of the volatile amine 2-dimethylaminoethanol in the brain of man and pig and in other biological material[1,2]. Bremer and Greenberg's[3] report on its presence in rat-liver phospholipids has substantiated our findings. During our investigation several volatile amines—besides the previously identified volatile bases ammonia[4] and 2-aminoethanol[5]—were isolated from brain tissue and we became interested in analysing the brain for other normally occurring volatile amines.

Brain tissues from cat, pig and man, processed 1, 30 and 360 min. after death, respectively, were used. The extraction was carried out by three different methods to get a more accurate picture of the amines present. Accordingly, the tissue was extracted 3–4 times with either 80 per cent ethanol, adjusted to *p*H 2 with hydrochloric acid (EtOH–HCl), as described earlier[2]; or with acetone followed by 80 per cent ethanol (acetone–EtOH); or with 5 per cent trichloracetic acid. The supernatant contained the unbound or free amines; the precipitate, the bound amines. Since we were primarily interested in the unbound amine content of the brain, most of the following procedures involved only the supernatant fractions. In the case of the trichloracetic acid treatment the aqueous solution was extracted with ether and passed through an anion exchange column ('Dowex 2', Cl^- form), in order to remove the trichloracetic acid. The supernatants were individually concentrated *in*

vacuo and subjected to a fractional steam distillation in saturated barium hydroxide solution or, in the case of the trichloracetic acid extract, in saturated sodium carbonate solution. The distillation and the subsequent procedures, used for obtaining the volatile amine fractions as hydrochlorides, were the same as previously reported[2]. To liberate the bound amines from human and pig brain tissue the (EtOH–HCl) precipitate was hydrolysed in saturated aqueous barium hydroxide and the bases fractionated according to the procedure described[2].

The different fractions were analysed by gas-chromatography, paper-electrophoresis and paper-chromatography. Gas-chromatography was performed with argon as the carrier gas and an ionization detector which has a usable sensitivity of 10^{-10}–10^{-11} mole. The insensitivity of the ionization detector to water allowed us to analyse the free amines in aqueous solution. The free amines were released from their hydrochloride salts with 2 *N* aqueous sodium hydroxide and injected into the gas-chromatograph, where they were separated by a carbowax-on-alkaline-chromosorb column[2]. The paper-electrophoretic and paper-chromatographic separations were carried out as previously described[2], using the hydrochloride salts of the amines.

The gas-chromatograms of the unbound amine fractions from the EtOH–HCl and acetone–EtOH extracts of pig brain and the trichloracetic acid extract of cat brain, as well as the bound amine fractions from pig brain (EtOH–HCl), showed quite similar patterns. 2-Dimethylaminoethanol, 2-aminoethanol and ammonia were present in these fractions plus 4–8 as yet unidentified amines. One of these amine-peaks recurs in the gas-chromatograms of all specimens and indicates the presence of 2-methylaminoethanol. Extracts from a human brain (EtOH–HCl) showed in the unbound and bound amine fractions an additional peak in large amounts which could be identified as 2-diethylaminoethanol.

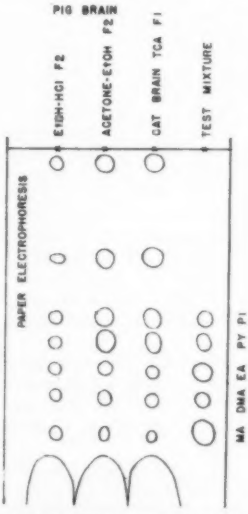

Fig. 1. Paper electrophoretic separation of brain fractions and test mixture. The migration values (where cysteine has a migration value of 0 and methylamine of 1) and dye reactions of the amines are: methylamine (MA) 1·0, ninhydrin red-violet; dimethylamine (DMA) 0·90; ninhydrin red-violet; ethylamine (EA) 0·79, ninhydrin blue-violet; pyrrolidine (PY) 0·69, ninhydrin yellow, isatin blue; piperidine (PI) 0·59, ninhydrin violet, red fluorescence under ultra-violet light

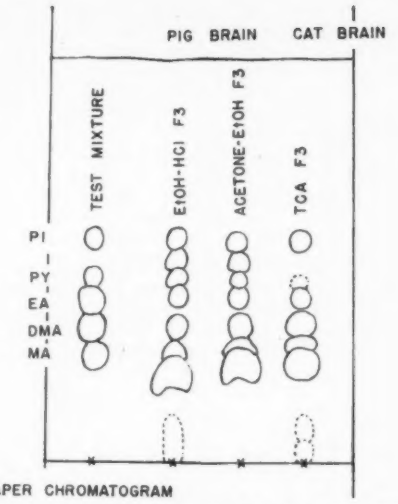

Fig. 2. Paper chromatographic separation of brain fractions and test mixture. The migration values are: methylamine (MA) 0·27; dimethylamine (DMA) 0·32; ethylamine (EA) 0·40; pyrrolidine (PY) 0·44; piperidine (PI) 0·53. The dye reactions were the same as in Fig. 1

It was learned later that the patient whose brain was analysed received treatment with 110 mgm. of procaine (in the form of procaine penicillin) every 6 hr. prior to death. This probably accounts for the large amount of 2-diethylaminoethanol present. Also, the occurrence of this substance in the bound form suggests the possibility of its incorporation into phospholipids.

Figs. 1 and 2 represent the paper-electrophoretic and paper-chromatographic separation of the pig and cat brain fractions, obtained by the different extraction procedures. It can be seen that these fractions are almost identical. By comparison with known amines, five of the spots could be identified as methylamine, dimethylamine, ethylamine, pyrrolidine and piperidine. These five substances were present in the free amine fractions of the EtOH–HCl and acetone–HCl extracts from pig brain and in the free amine fractions from cat brain, obtained by trichloracetic acid extraction. In the bound fractions from pig brain (EtOH–HCl) methylamine, dimethylamine, ethylamine and pyrrolidine were identified. The human brain (EtOH–HCl extraction) contained in the free amine fractions methylamine, dimethylamine, ethylamine and piperidine; in the bound amine fractions piperidine was missing. The migration values and dye reactions of the substances from the brain extracts and the mixture of reference compounds coincided in the chromatographic and electrophoretic systems. In addition, the reference compounds were mixed with the brain fractions and the chromatographic and electrophoretic concordance was determined from the increased spot intensities. The results were further substantiated by a two-dimensional electrophoretic-chromatographic separation on one paper[6]. Besides the identified amines this separation revealed a substantial number of unidentified amines. The largest number was found in the EtOH–HCl extract of pig brain, 12 unknown spots in the free amine and 11 in the bound amine fractions. This might be due to the relatively large amount of tissue analysed, which allowed detection of amines present in low concentrations.

Table 1. Approximate Amounts of Unbound Volatile Amines in Brain (in Free Amine Form)

	Pig brain		Cat brain
	EtOH–HCl (μgm./kgm.)	Acetone–EtOH (μgm./kgm.)	TCA (μgm./kgm.)
Methylamine	110	40	790
Dimethylamine	550	290	2,460
Ethylamine	90	60	360
Pyrrolidine	20	10	20
Piperidine	30	180	840
Dimethylamino-ethanol	45	40	80

Table 1 gives the approximate amounts of the volatile amines which we found in brain tissue. The value for 2-dimethylaminoethanol is most exact because it was calculated from the peak which appeared on the gas-chromatograms, whereas the remaining substances have been estimated from the density of the spots as they appeared in paper-chromatography and paper-electrophoresis. Furthermore, since much more pig brain than cat brain was assayed, the figures for pig brain may be more representative than those for the cat brain.

Further work is planned to confirm these results with methods which exclude possible hydrolysis of unstable substances and to learn more about the significance of the occurrence of these substances in the various organs of animals under varying physiological conditions.

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Conrad G. Honegger*
Ruth Honegger

Department of Pharmacology,
Division of Basic Health Sciences,
Emory University,
Atlanta 22, Georgia.

* Visiting assistant professor on leave of absence from Wissenschaftliches Laboratorium der Psychiatrischen Universitaetsklinik und der Neurologischen Universitaets-Poliklinik Basel, Mittlere Strasse 91, Basel, Switzerland.

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Methanesulphonic Acid Derivatives of Central Stimulant Drugs

Among the substances recently introduced in the therapy of mental depression which stimulate the central nervous system, iproniazide (1-*iso*nicotinoyl-2-*iso*propyl-hydrazine) has been the drug longest under investigation[1]. Its stimulating properties have been discovered by studying this drug as a tuberculostatic agent; and during this work toxic psychoses have been observed as side-effects.

A great number of hydrazine derivatives tested afterwards reacted in the same way and showed a more or less pronounced inhibition on the monoamine oxidase, an enzyme involved in the metabolism of serotonin, epinephrine and other amines. These substances unfortunately produce a wide variety of side-effects, so that many physicians refuse to use them.

Table 1. Toxicity (*LD*50)

Drug	Administered orally (rats) (mgm./kgm.)	Administered intravenously (mice) (mgm./kgm.)
(1) Iproniazide	365	820
(2) 2-Hydrazino-propane hydrochloride	80	315
(3) 1-Phenyl-2-propyl-hydrazine hydrochloride	13	—
(4) Sodium salt of methanesulphonic acid of (2)	330	500
(5) Sodium salt of methanesulphonic acid of (3)	56	—

Table 2. Inhibition *in vivo* of the Monoamine Oxidase Dose of drug producing 50 per cent inhibition, administered endoperitoneally 6 hr. before the animals were killed (ref. 6)

Drug	Brain (mgm.)	Liver (mgm.)
(1) Iproniazide	6·2	1·2
(2) 1-Phenyl-2-propyl-hydrazine hydrochloride	0·4	0·45
(3) Sodium salt of methanesulphonic acid of (2)	0·70	0·70
(4) Sodium salt of methanesulphonic acid of 2-hydrazino-propane	2·45	0·61

Table 3. Brain Serotonin-Levels of Rats after Administration of Drugs 6 hr. before the Animals were Killed (ref. 6)

Drug	Dose (mgm./kgm.)	No. of animals	Brain serotonin (γ/gm. fresh weight ± S.E.)
Control	—	15	0·470 ± 0·021
(1) Iproniazide	100	10	0·994 ± 0·038
(2) Sodium salt of methanesulphonic acid of 2-hydrazino-propane	114	10	1·400 ± 0·045
(3) 2-Hydrazino-propane hydrochloride	61	10	1·860 ± 0·065

In order to get a therapeutically more suitable drug with a lower toxicity we adopted a method which has already given good results with other drugs[2], namely, transformation into methanesulphonic acid derivatives. Thus we transformed 2-hydrazino-propane[3] and 1-phenyl-2-hydrazino-propane[4] (both substances are considerably more active than iproniazide) into the above derivatives. We treated 2-hydrazino-propane and 1-phenyl-2-hydrazino-propane with formaldehyde (40 per cent) and then with sulphur dioxide to obtain the corresponding methanesulphonic acids, which we neutralized with sodium bicarbonate to form the sodium salt of methanesulphonic acid of 2-hydrazinopropan (I) and the sodium salt of methanesulphonic acid of 1-phenyl-2-hydrazino-propane (II).

The sodium salts have no definite melting point, but the acid corresponding to (I) melts at 158°, that of (II) at 136–138°[5]. Analysis of the acid of (I) gave:

Calculated for $C_4H_{12}N_2SO_3$: C, 28·55; H, 7·19; N, 16·42; S, 18·50.

Found: C, 28·33; H, 7·16; N, 16·42; S, 18·50.

Analysis of the sodium salt of (I) gave:

Calculated for $C_4H_{11}N_2SO_3Na.2H_2O$: N, 12·37.

Found: N, 12·85.

Analysis of the sodium salt of (II) gave:

Calculated for $C_{10}H_{15}N_2SO_3Na.3H_2O$: N, 8·76; S, 10·02.

Found: N, 8·40; S, 9·82.

Table 1 shows a lower toxicity compared to the initial substances, and Tables 2 and 3 show in the same time a high activity with regard to the inhibition of monoamine oxidase and to the increase of the brain serotonin.

Th. Bersin[7] has attributed this surprising change of the pharmacological properties of the methanesulphonic acid derivatives to the transformation of a cationic drug into an anionic one:

$$RNHNH_3^+ \rightarrow RNH.NH.CH_2SO_3^-$$

This transformation presumably changes the penetration and excretion of the drug.

W. Logemann
F. Lauria
D. Artini

Chemical Research Laboratory,
Istituto Carlo Erba per
Ricerche Terapeutiche,
Milan.

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Release of Iron from Conjugates in Foods

Iron in food of both animal and plant origin must be released from its conjugates before it can be absorbed from the gastro-intestinal tract. There is also evidence that it must be in the reduced ferrous state before assimilation. The total iron and the percentage easily available as ferrous iron are therefore both important in assessing the dietary value of an iron-containing food, and for this reason most tables of food values show the total iron and that amount which reacts with α-α′-dipyridyl. These measurements, however, do not recognize the possible effects of cooking and of intestinal enzymatic digestion in releasing iron from its conjugates.

Kaldor demonstrated that 12–13 per cent of the total iron in hæmoglobin is liberated by acid peptic digestion of heat-denatured blood, whereas only approximately 0·1 per cent could be extracted with saline from uncooked blood[1]. Brading, Kaldor and George also showed that more iron was absorbed by rats from cooked than from uncooked blood administered into the stomach[2]. These observations have been based solely on hæmoglobin, although Moore and Dubach have shown that iron-59 incorporated into various foods is absorbed from the gastro-intestinal tract[3]. The part played by acid peptic digestion *in vitro* in liberating iron from cooked foods has now been extended to a number of other materials.

The total iron content was determined on acid digests of the foods. Aliquots of calves' liver and flour were heated to 95° C. for 30 min., and of silver beet and sultanas to 100° C. for 30 min. They were then homogenized in the water or saline in which they had been heated. One aliquot of each homogenate was incubated with water or saline at 37° C. for 60 min. and another with pepsin at a final concentration of 0·64 per cent and hydrochloric acid at a concentration of 0·32 *N* (*p*H 1·5). The supernatant of the first treatment is referred to as the neutral extract and of the second as the acid peptic digest. The iron content was determined in each case, using the ortho-phenanthroline method described by Kaldor[4].

The importance of cooking hæmoglobin is seen from the results in Table 1, which also demonstrate the role of peptic digestion and the variability with which iron is released from different foods. Higher percentages are liberated from liver rather than from hæmoglobin, and the iron from the liver was shown to be derived more from the tissue iron (hæmosiderin and ferritin) rather than from the contained blood. The plant products yielded greater percentages than did hæmoglobin, but smaller absolute amounts per gram because of the lower total iron content. Phytic acid in silver beet did not prevent liberation of iron, but the amount of iron released was greater from old than from young leaves, and greater from stored than from fresh leaves.

These findings suggest a more rational basis for the compilation of dietary tables of iron values. However, the amount of iron released by peptic digestion of cooked food is not the only factor determining the amount of iron actually absorbed following ingestion of the food. The role of the other enzymes present in the gastro-intestinal tract has not been investigated. The needs of the body also have some regulating

Table 1. Extraction of Iron from Foods

Food	Treatment		No. of assays	Total iron content (mean)	Iron liberated (mean)	Per cent total iron liberated (mean)
Blood	Raw	saline extraction	2	524·4 μgm. Fe/ml.	5·8 μgm. Fe/ml.	1·1
		acid peptic digestion	6	518·9 ,,	12·5 ,,	2·4
	Cooked	saline extraction	5	535·7 ,,	8·0 ,,	1·5
		acid peptic digestion	15	529·6 ,,	53·5 ,,	10·1
Liver	Cooked	saline extraction	6	60·5 μgm. Fe/gm.	1·6 μgm. Fe/gm.	2·6
		acid peptic digestion	12	66·6 ,,	34·4 ,,	51·6
Plain flour	Cooked	water extraction	21	10·3 ,,	1·0 ,,	9·7
		acid peptic digestion	24	10·3 ,,	3·5 ,,	34·1
Silver beet	Cooked	saline extraction	6	18·3 ,,	3·0 ,,	16·5
		acid peptic digestion	36	20·5 ,,	13·2 ,,	64·4
Sultanas	Raw	saline extraction	9	17·5 ,,	0·8 ,,	4·7
		acid peptic digestion	10	18·4 ,,	8·6 ,,	46·9
	Cooked	saline extraction	11	14·8 ,,	2·4 ,,	16·1
		acid peptic digestion	9	17·3 ,,	10·7 ,,	62·1

effect on absorption but the mechanisms have not yet been elucidated.

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Rona Sanford

New South Wales Red Cross
Blood Transfusion Service,
1 York Street,
Sydney.

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Optical Rotation and Configuration of Poly-L-Tyrosine

In a recent communication[1], Downie, Elliott and Hanby report that the optical rotatory dispersion of poly-L-tyrosine is unusual and, as a result, its configuration cannot be assigned by analogy with polypeptides having normal dispersion characteristics. However, they interpret their data, together with unspecified infra-red spectral and X-ray diffraction observations of films, as indicating that "it is more probable that in dilute solutions in pyridine, in dimethyl formamide, and in aqueous alkali, poly-L-tyrosine is in a random coil configuration". Our study of this problem has led to somewhat different conclusions: that the configuration in pyridine and dimethyl formamide is that of the α-helix, whereas in alkaline solution the configuration is dependent on *p*H, indicative of the random-coil configuration. The basis of the configurational assignments will be published elsewhere in detail, but we wish to summarize them here and report the optical rotatory characteristics of the two forms.

Poly-L-tyrosine samples were prepared by modification of the Katchalski–Sela[2] procedure and shown to have molecular weights in the range 30,000–40,000 by combined sedimentation and viscosity measurements. In this molecular-weight range such measurements alone cannot establish configuration. Flow-birefringence measurements did, however, show that in dimethyl formamide/glycerol (3 : 1 by volume) the polymer exhibited strong positive birefringence. The same polymer sample when dissolved in aqueous alkali (*p*H 12·5, 1 *M* sodium chloride) – glycerol solution of equivalent viscosity failed to show any birefringence.

The specific rotation of poly-L-tyrosine was measured in the *p*H range where it is soluble in aqueous solution, that is, above *p*H 10·6. The results, shown in Fig. 1, indicate a sharp fall in specific rotation coincident with the ionization of the tyrosine residue. The specific rotation of tyrosine itself changes only a few degrees in this same *p*H range. Since large changes in the optical rotation of poly-L-glutamic acid[3] and poly-L-lysine[4] were accompanied by a helix-coil transition, it was inferred that the same was true for poly-L-tyrosine. This was tested by measuring the sedimentation constant and specific viscosity as a function of *p*H at two different ionic strengths (0·2 and 1·0). Below *p*H 11·3, there was no dependence on ionic strength, indicating a rigid configuration. Above this *p*H the values were increasingly dependent on ionic strength, as would be expected for a flexible coil configuration. This

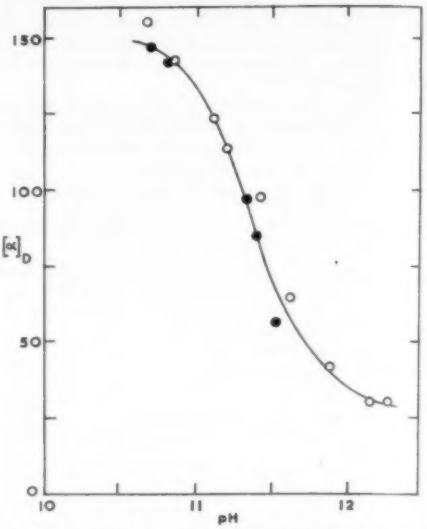

Fig. 1. Dependence on *p*H of specific optical rotation (at 589 mμ) of poly-L-tyrosine. All measurements were made in the 0·2 *M* sodium chloride. ● and ○ denote different polymer samples

supporting evidence leads to the conclusion that the configuration is helical near the solubility limit and randomly coiled beyond *p*H 12.

One can expect to observe the same helix-coil transition in organic solvents as the proportion of weak to strong hydrogen-bonding solvents is increased. This was found by measuring the specific rotation, $[\alpha]_D$, as a function of the concentration of dichloroacetic acid in dichloroacetic acid – dimethyl formamide mixtures[6]. The value of $[\alpha]_D$ fell sharply from 190° to 40° in the vicinity of 75 per cent dichloroacetic acid[6]. Since the strong birefringence indicated the helical form in dimethyl formamide, and since dichloroacetic acid has been shown to induce the solvated, random-coil configuration[7] and since, furthermore, the $[\alpha]_D$ values in these two solvents match the values of the two similarly assigned configurations in aqueous solution, it can be concluded that the α-helical configuration exists in dimethyl formamide, pyridine and at *p*H 10·7 in aqueous solution, while the random-coil configuration exists at high concentrations of dichloroacetic acid and above *p*H 11·8.

Having made this configurational assignment, it is of interest to examine the rotatory dispersion of the two forms in order to assess the magnitude of the perturbation produced in dispersion constants by the introduction of a strong chromophoric group close to the peptide bond chromophore. The relevant data for each configuration in two quite different solvents are listed in Table 1. The constant λ_0 was taken as 212 mμ in determining b_0 and the resulting plots were linear over the wave-length range investigated (450–700 mμ).

It is seen that each of the three constants has characteristic values for the two configurations. $[\alpha]_D$ for the helical form is more positive than the coil form by 120–150°. When data on this difference for six other, normally behaving, polypeptides is averaged and corrected to the residue weight of tyrosine, a predicted difference of only 50° is found.

Table 1. ROTATORY DISPERSION CONSTANTS OF POLY-L-TYROSINE

Solvent	$[\alpha]_D$	λ_0	b_0
0·15 *M* NaCl, *p*H 10·85	+ 150°	237 mμ	+ 540°
0·15 *M* NaCl, *p*H 11·20	+ 103	263	+ 520
0·15 *M* NaCl, *p*H 11·88	+ 38	316	+ 480
0·15 *M* NaCl, *p*H 12·27	+ 28	330	+ 450
Pyridine	+ 189	230	+ 560
Dimethyl formamide	+ 184	235	+ 550
Dichloroacetic acid			
" with 20 per cent dimethyl formamide	+ 73	330	+ 470
" with 4 per cent dimethyl formamide	+ 36	—	—

Thus, the sign of the difference for poly-L-tyrosine is normal, but the magnitude is about 2·5 times that normally observed.

The constant λ_0 usually has a value of about 210 mμ for the coil form, and increases to 300 mμ for the helix form. The behaviour found here is approximately the inverse.

The constant b_0 normally has a value of about −630 for the helical form and zero for the coil form. For poly-L-tyrosine we find instead positive values for both, and little dependence on configuration.

From this it is clear that the proximity of the tyrosine residue to the peptide bond greatly modifies the optical rotatory properties of both the helical and the coil forms. The close coupling between the two chromophores in either configuration, plus the fact that the primary helical arrangement of peptide bonds necessarily imposes a second helical arrangement on the chromophoric side-chains (tyrosine), introduces sufficient new pair-wise interactions to make the contribution of the original primary helix of peptide bonds to the optical rotatory properties essentially unrecognizable.

It might be conjectured that some of the changes in dispersion constants may be due to the helical form having the opposite screw sense to that normally occurring[7]. However, co-polymer studies to be described elsewhere rule out this possibility.

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JAMES D. COOMBES
E. KATCHALSKI
PAUL DOTY

Department of Chemistry,
Harvard University,
Cambridge, Mass.
Dec. 2.

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A New Metabolite of Chloramphenicol

THE metabolism of chloramphenicol has been studied extensively in animals and in human subjects, with the recognition of the glucuronide as the principal metabolic product[1]. In addition, a small amount of the amino diol hydrolysis product and free chloramphenicol were found in the urine[1,2]. However, little attention has been given to the metabolism of this compound where deficiencies exist in the conjugating mechanism. The normal adult cat has been reported to be deficient in its capacity to form glucuronides with a number of synthetic substrates[3], and newborn animals generally have a low order of activity in the enzyme systems responsible for detoxication processes[4]. Consequently, the metabolism of chloramphenicol was investigated in the cat and in newborn human infants.

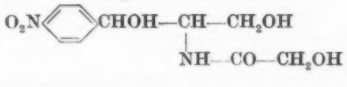
Following oral administration of chloramphenicol palmitate, the urine of adult cats and newborn infants was examined by paper chromatography. The solvent system was water-saturated butanol containing 2·5 per cent phenol and 2 per cent pyridine[1]. Strips of Whatman No. 1 paper were used in the descending method, and nitro compounds were detected by reduction with titanous chloride, diazotization and coupling as described elsewhere[1]. Aromatic nitro compounds were found in positions corresponding with chloramphenicol, the amino diol hydrolysis product of chloramphenicol, and the glucuronic acid conjugate. In addition, a fourth band located at R_F 0·70–0·75 was observed in cat urine and in the urine of newborn infants. A similar band was later observed in the urine of normal rats.

The presence of a glucuronide band in cat urine was of considerable interest in view of earlier reports concerning the absence of conjugation with phenolic compounds[3]. It is possible that conjugation of the terminal aliphatic alcohol group of chloramphenicol with glucuronic acid may occur through a different mechanism. The glucuronide nature of the product was confirmed by spraying the paper chromatograms with a solution of β-glucuronidase. After incubation for a few hours in a moist atmosphere, the strips were placed upon agar plates seeded with *Sarcina lutea*. Antimicrobial activity was found in the position corresponding with the chloramphenicol–glucuronide band, which showed no antimicrobial activity prior to hydrolysis.

The appearance of a new metabolite of chloramphenicol was also of interest because of its potential toxicity. This compound was isolated from cat urine, using a combination of methods involving adsorption on to charcoal, fractional elution with acetone–water mixtures, 24-plate counter-current extraction with a two-phase solvent system consisting of 270 ml. water, 30 ml. *iso*propanol and 300 ml. ethyl acetate, and by paper chromatography. The final crystalline product had an off-white colour and gave a melting point of 155–156° C. (uncorr.). It showed 4 per cent of the antimicrobial activity of chloramphenicol against *Shigella sonnei* in turbidimetric assays. $E(1\%, 1\text{ cm.}) = 367$ at 274 mμ (ethanol).

Analysis: Calc. for $C_{11}H_{14}N_2O_6$ (270·24): C, 48·89; H, 5·22; N, 10·37. Found: C, 49·26, 49·34; H, 5·38, 5·34; N, 10·44.

The analytical results are in good agreement with the structure for D(−)*threo*-1-*p*-nitrophenyl-2-hydroxyacetamido-1,3-propanediol:



The metabolite is a glycolic acid amide analogue of chloramphenicol, apparently resulting from dehalogenation and reduction of the dichloracetate group in the parent compound. This analogue was prepared by synthesis, using the acid chloride procedure described by Rebstock[5]. The product showed the

same melting point and mixed melting point as the metabolite from cat urine. $E(1\%, 1\,cm.) = 375$ at 274 mμ (ethanol). The infra-red absorbance spectra of the two preparations were identical, and they showed the same solubility characteristics in the counter-current extraction system already described, as well as having the same R_F values in paper chromatograms, and the same antimicrobial activity. The metabolite present in the urine of newborn human infants was also isolated by the same procedure, and proved to be identical with the product from cat urine in its counter-current distribution, paper chromatography, and antimicrobial activity.

Preliminary acute toxicity data obtained by intravenous administration of the synthetic product gave an $LD50$ value of 2,950 ± 81 mgm./kgm. in rats, and 2,950 ± 72 mgm./kgm. in mice, indicating that the metabolic product has a very low order of toxicity.

W. A. Dill
E. M. Thompson
R. A. Fisken
A. J. Glazko

Research Laboratories,
Parke, Davis and Co.,
Detroit, Michigan.

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Effect of Glycosylation on the Enzymic Oxidation and Translocation of Flavonoids

There is a possibility that a planar structure may be one of the factors which determines the ability of a flavonoid to function as a substrate for plant polyphenoloxidases. It is also possible that a planar structure may adversely affect the ease of translocation of flavonoids in vegetable tissues.

As a result of an extensive study of the paper chromatographic behaviour of phenolic substances it has been established that all flavonoids with a planar structure have zero R_F in water, or in aqueous solvents containing up to 2 per cent of acid[1]. The molecular model in Fig. 1 illustrates the planar structure of quercetin. Other polynuclear phenols with 'flat' molecules, for example, ellagic acid, resveratrol and galloflavin, have also been shown to have zero R_F values in water. It is known that cellulose absorbs phenolic substances, and it appears that when a molecule contains two phenolic rings in the same plane the adsorption by cellulose is sufficient to prevent elution of the adsorbed substance by aqueous solvents.

The R_F values in aqueous solvents of substances such as gallic and caffeic acids are significantly increased by combination with glucose and quinic acid.

	R_F in 2 per cent acetic acid
Gallic acid	0·44
Glucogallin	0·64
Caffeic acid	0·25
Chlorogenic acid	0·56

As the R_F values in water of flavonol-3-glycosides are significantly greater than zero[2], it was originally assumed that the increased R_F was due to combination with water-soluble sugar residues. However, it has been found that both kaempferol-4′-arabinoside and quercetin-4′-glucoside have zero R_F values, and Geissmann has also recorded zero R_F in water for quercetin-7-glucoside (quercimeritrin)[3].

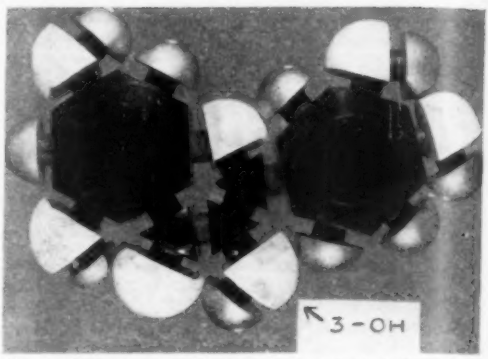

Fig. 1. Planar structure of the quercetin molecule built up from Courtauld's atomic models. Note that the hydrogen atom of the 3-hydroxyl group can only just be accommodated in the planar structure

The introduction of a sugar residue into the 4′ or 7 positions of a flavonol does not destroy the planarity of the molecule. On the other hand, as will be apparent from an inspection of the molecular model of quercetin, substitution of a large group in the 3-position cannot be achieved without a loss in planarity. Glycosylation only increases R_F values in aqueous solvents if in the 3-position, so that it appears that the increase is a consequence of loss of planarity and not to combination with a large water-soluble group.

It was shown by Baruah and Swain that potato polyphenoloxidase catalysed the oxidation of quercetin, myricetin and luteolin, but that the 3-glucosides of quercetin and myricetin were not oxidized[4]. This confirmed and extended earlier work by Roberts and Wood[5]. These results suggest that plant oxidases act preferentially upon flat molecules, a planar structure facilitating adsorption by the active enzyme surface. One might therefore anticipate that glycosides of luteolin, quercetin and myricetin might function as substrates so long as the sugar group was not in the 3-position, and it has been shown by Baruah and Swain[4] that the 7-diglucoside of luteolin (chærophyllin) undergoes oxidation in the presence of the potato oxidase. However, it was also stated that quercimeritrin was not oxidized, and this is inconsistent with the hypothesis advanced.

Samples of these two flavonoids were made available by Dr. Swain, and, as expected, it was found that chærophyllin had zero R_F in 2 per cent acetic acid. However, the substance labelled quercimeritrin was almost certainly inaccurately described, for it was resolved by two-way paper chromatography into at least five components, only one of which had zero R_F in aqueous solvents. It has been confirmed in this laboratory that an authentic specimen of quercimeritrin has an R_F of zero in 2 per cent acetic acid, and it may be safely concluded that Baruah and Swain's findings for quercimeritrin are incorrect. In summarizing the relation between substrate activity and R_F values in aqueous solvents (Table 1), results for quercimeritrin have not therefore been included.

The strong adsorption of flavones and flavonol aglycones by cellulose may well restrict their mobility

Table 1. Substrate Activities and R_F Values in Aqueous Solvents of Flavonoids

Substrate	Structure	R_F value	Oxidized by enzyme
Quercetin	Planar	0·00	Yes*
Quercetin-3-rhamnoside	Non-planar	0·19	No
Quercetin-3-rhamno-glucoside	Non-planar	0·29	No
Myricetin	Planar	0·00	Yes
Myricetin-3-rhamnoside	Non-planar	0·23	No
Luteolin	Planar	0·00	Yes
Luteolin-7-diglucoside	Planar	0·00	Yes

* Roberts and Wood (ref. 5) stated that quercetin was not oxidized by the tea oxidase. This was an inaccurate observation due to the very low solubility in water of quercetin.

in plant tissues, and it is suggested that glycosylation in the 3-position is necessary for translocation to take place. A paradoxical situation may now arise, for the planarity of the molecule, which is necessary for any physiological activity dependent upon enzymic oxidation, will hinder translocation. On the other hand, the more mobile glycoside may be physiologically inactive unless there is a glycosidase present to regenerate the active aglycone. Considerations such as these may also be relevant to the activity of certain flavonoids on vascular resistance or capillary permeability in animals[6,7].

I wish to thank Dr. T. Swain for helpful discussions, and Dr. D. E. Hathway and Profs. R. D. Haworth and L. Hörhammer for specimens of resveratrol, gallo-flavin and flavonol-4′-glycosides. Thanks are also due to the Indian Tea Association for permission to publish this communication.

E. A. H. Roberts

Indian Tea Association (London),
Butler's Wharf,
Lafone Street, London, S.E.1.

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Need for Inorganic Phosphate in Oxidations stimulated by Dinitrophenol

Recently we have reported, in disagreement with the widely accepted view, that the maximal rate of oxygen uptake by respiring rat-liver or rat-heart mitochondria with glutamate as substrate is not obtained with 2,4-dinitrophenol unless phosphate is also present[1]. With succinate as substrate, on the other hand, the same initial rate of oxygen uptake was found with dinitrophenol as with inorganic phosphate. We concluded that a link in the respiratory chain between glutamate and cytochrome *b* required phosphate even in the presence of dinitrophenol, a conclusion which seemed to have important implications for theories of the mechanism of the uncoupling of oxidative phosphorylation by dinitrophenol.

We now wish to report that further studies have provided evidence that dinitrophenol can replace inorganic phosphate in the respiratory chain. There is, therefore, no need to modify our earlier view[2] that dinitrophenol reacts with an energy-rich intermediate in oxidative phosphorylation formed prior to the intervention of inorganic phosphate.

The new evidence we have obtained is :

(1) Substrates, such as malate and β-hydroxybutyrate, which operate through nicotinamide-adenine dinucleotides, are oxidized at the same rate in the presence of 2,4-dinitrophenol alone as with dinitrophenol and inorganic phosphate together.

(2) The results with glutamate are explained by the finding that a large part of the measured oxygen uptake with this substrate is not concerned with the oxidation of glutamate to α-ketoglutarate, but with the further oxidation of the α-ketoglutarate. This is shown by the fact that the α-ketoglutarate (μmoles) found in the presence of phosphate was less than 1 per cent of the oxygen uptake (μatoms). Malonate (0·02 *M*) inhibited by 70 per cent the rate of oxygen uptake with liver mitochondria. The requirement for inorganic phosphate for maximal rates of oxygen uptake is explained by the fact that dinitrophenol cannot replace inorganic phosphate in the 'substrate-linked' phosphorylation step associated with α-keto-glutarate oxidation[3].

The rapidity with which the α-ketoglutarate formed from glutamate is oxidized by mitochondrial preparations was unexpected. It is clear that in the absence of an inhibitor of α-ketoglutarate oxidation, glutamate is not a suitable substrate for studying oxidative phosphorylation and respiratory control associated with the oxidation of reduced nicotinamide–adenine dinucleotides.

We wish to thank Prof. F. Lynen for a stimulating discussion which led us to examine more closely the products of oxidation of glutamate. This work was supported by a grant from the Netherlands Organization for National Health Research T.N.O. and the Koningin Wilhelmina Fund for Cancer Research.

P. Borst
E. C. Slater

Laboratory of Physiological Chemistry,
University of Amsterdam.

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PHARMACOLOGY

Histamine Sensitivity in Children after *Pertussis* Infection

It has been shown in recent years that mice and rats are rendered hypersensitive to the action of histamine, 5-hydroxytryptamine and anaphylactic sensitization by pre-treatment with *Hæmophilus pertussis* vaccine[1-4]. These properties are present only in organisms in the smooth phase and are not shared by other members of the *Hæmophilus* family[5,6]. The sensitizing action is not seen in the guinea pig and the rabbit; on the other hand, there is some evidence that these two species are rendered slightly more resistant to histamine after such treatment[7]. Rats and mice are relatively resistant to histamine and anaphylactic shock, whereas guinea pigs and rabbits are susceptible. It was therefore of interest to study whether the reaction in man follows a similar pattern to the former two species or the latter. Recently there was an epidemic of whooping cough in Darbhanga and advantage was taken of this for such a study.

Histamine sensitivity was tested by the method developed by Jha in this laboratory. Briefly, it consists of placing on the fore-arm a lint soaked in an aqueous solution of histamine acid phosphate (10^{-5} conc. as base) and another in *N* saline. The anode of a 6-V. dry battery is placed on the histamine lint and the cathode on the other and current is allowed to pass for exactly 2 min. The reaction is read in 15 min. time. The normal reaction consists of erythema and occasional papules which never cover more than a quarter of the area of application, and is arbitrarily classified as (+). When papules cover half the area the reaction is read as (++) and when more than three-quarters are involved as (+++). A wheal formation is indicative of a marked hypersensitivity and is designated as (++++). By giving a score 2 to each (+), the average reaction can be expressed as a percentage of the maximal reaction possible. In this system of scoring, normal reaction is between 0 and 20 per cent, and values above 50 per cent represent hypersensitivity.

Sensitivity to 5-hydroxytryptamine, and the histamine liberator compound 48/80 can also be studied similarly.

Thirty children in the typical stage of 'whoop' in the age group 1–10 years were selected for the study, and the sensitivity to histamine, 5-hydroxytryptamine and compound 48/80 was determined separately. It was found that nearly all the patients were maximally sensitive to histamine (average reaction 88 per cent), and slightly sensitive to 5-hydroxytryptamine (average reaction 40 per cent). Wheals developed after histamine iontophoresis in majority of the patients, but were never encountered after 5-hydroxytryptamine. The patients failed to exhibit sensitivity to compound 48/80 (average reaction less than 10 per cent).

Thus it is seen that infection with *Hæmophilus pertussis* produces a condition in children similar to that noted in rats and mice. It is difficult to say at present whether such children are more susceptible to allergic reactions as well.

It has been reported that the anaphylactoid reaction (œdema) in rats is mediated through 5-hydroxytryptamine[8]. Such a conclusion may not be true for man, for although histamine in many cases produced an urticarial wheal, 5-hydroxytryptamine failed to do so when applied in a similar concentration. It may be noted that 5-hydroxytryptamine is 200 times as potent as histamine in producing the œdema reaction in the rat[9].

The fact that, even in patients maximally sensitive to histamine, compound 48/80 failed to produce significant reaction leads to the indirect inference that histamine content of the skin of children is low, particularly as when the experiments were repeated in adults, in many cases, typical reactions were obtained.

R. K. Sanyal

Department of Pharmacology,
Darbhanga Medical College,
Laheriasarai P.O., India.

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Interactions between Pharmacodynamic and Placebo Effects in Drug Evaluations in Man

In the course of the development of a method sufficiently sensitive to evaluate the effects of small doses of tranquillizers in man, evidence of interactions between pharmacodynamic and placebo effects of drugs was adduced. Since placebo action (that is, the response to the act of taking medication as distinguished from the pharmacodynamic action of the medicament) must be taken into account in analysing human reactions to drugs, we feel that our findings have implications of general interest as well as of practical importance in designing methods for observing and evaluating the effects of drugs in man.

The influence of competitive mental work (a variant of the Stroop test[1]) on high-amplitude finger tremor and its modification by sedative drugs was studied in 12 male medical students. To control bias as well as placebo effects, dummy medication[2] in a capsule of identical appearance was administered in a classic double-blind arrangement[3]. To evaluate the extent of the placebo influence on the results, the test was also conducted with neither drug nor dummy. The tests were randomized.

The pertinent findings may be briefly summarized. After dummy medication had been given, the rate of high-amplitude finger tremor rose during the test from an average of 60 to 198 per min. ; after 100 mgm. of pentobarbital sodium, it rose to 162 ; when neither drug nor dummy had been given, it rose to only 152 per min. By the sign test, statistical analysis indicated that each of the latter two is significantly smaller than the rate after the dummy capsule ($P < 0{\cdot}05$). However, the rate after 50 mgm. of pentobarbital did not differ significantly from that after the dummy.

Depending on the control used, the data may provide two diametrically opposed answers to the question of the effect of a sedative on high-amplitude tremor after competitive mental work. Thus, if the rate of tremor when neither drug nor dummy had been given is used as a control, 100 mgm. of pentobarbital is seen to have increased the tremor-rate. However, when the effect of 100 mgm. of pentobarbital is compared with the tremor-rate after a dummy, the drug appears to have decreased the tremor-rate. These results indicate that, with reference to the untreated, the drug made matters worse, whereas with reference to a placebo, the drug made matters better.

The increase in tremor-rate produced by 50 mgm. of pentobarbital is seen to fall within the range of the placebo effect. Consequently, the extent of the pharmacodynamic effect, if any, is uncertain. As a corollary, the placebo effect of the active agent is also uncertain, but it does confirm the stressful effect of the act of medicating.

The implications of these results are important for the clinical evaluation of drugs. The findings indicate that it is not always possible to anticipate the nature or the direction of placebo reaction in a particular circumstance. In this work, after it had been determined that the competitive mental work test did regularly and predictably increase the rate of high-amplitude finger tremor, it was presumed that the dummy would act as a placebo, that is, it would reduce the rate of high-amplitude finger tremor during the competitive mental work. However,

it was found that the dummy medication intensified high-amplitude finger tremor and, as a result, tremor-rate after a sedative was also greater than when no treatment was given.

It is obvious from these trials that difficulty may arise in defining the base-line for the estimation of pharmacodynamic actions in man, hence in making absolute measurements of drug action. In this study, because drug and placebo effects were opposed, drug action could be measured in terms of the extent by which the drug diminished the placebo reaction. In the situation in which drug effect is in the same direction, but exceeds placebo effects, the question of how to obtain an absolute measure of drug effect is a difficult one, for the results may not indicate whether the drug action supplements placebo effect or accompanies it. In other cases, in which the subtraction indicates no effect in excess of placebo effect, it does not rule out the possibility of the complete masking of drug effect. This also brings up the question of the sensitivity of the method requisite for drug evaluations since, as the relative proportions of placebo and pharmacodynamic actions in the total effect change, the ability of a method to discriminate between them, hence its sensitivity, also changes[4].

It is of especial interest, therefore, that a so-called 'negative' placebo effect is not necessarily an obstacle to the development of a useful and sensitive method of drug evaluation, for if the nature and the direction of placebo reaction, hence the base-line, can be identified, drug effect can be measured accurately enough; indeed, sometimes more accurately when they are opposed than when the effect of one obscures the effect of the other, as it may, when they develop simultaneously and in the same direction.

In the present instance, the dummy induced, not a typical placebo effect but, instead, a significant and useful stress, and the competitive mental work test merely provided an additional challenge, while the pharmacodynamic effect opposed both. It can be assumed that, regardless of how well they were re-assured that there was no hazard, the use of volunteer medical students[5] who knew that they were participating in a drug investigation led to the unexpected and uniform placebo response. It is possible that, were the subjects entirely unaware of participation in an investigation and, more specifically, of drug testing, both placebo and pharmacodynamic actions might have been in the same, instead of opposite, directions. However this may be, this circumstance may nevertheless provide a well-controlled, sensitized setting for the evaluation of the small doses of tranquillizers so commonly used in general practice.

The importance of 'negative' placebo effects in simulating toxic effects of drugs has been stressed in the literature[6]. In the present case, such an effect has been measured and has been shown to be regular and predictable. Because of the several varieties of placebo reactions as well as their potential for interaction with pharmacodynamic effects of drugs[7], this dynamic quality, which is inseparable from the act of drug administration in man, must be taken into account, and controls must be used which identify the direction as well as the magnitude of placebo effects. For this it may be necessary to use the unblinded 'no-drug-at-all' control to complement the usual identical dummies in the double-blind setting.

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WALTER MODELL
MARGARET GARRETT*

Department of Pharmacology,
Cornell University Medical College,
New York.

* Postdoctoral Fellow, U.S. Public Health Service Training Grant.

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ANIMAL PHYSIOLOGY

Relation of Eye Dominance and Eye Closure

IT is a well-known fact that while our vision is binocular, one eye (more often the right than the left) is in fact preferred or dominant in fixation and sighting. Much has been published on this subject, especially in its relation to handedness. We conjectured that if one eye is dominant in sighting, the other, that is, the non-dominant eye, would logically be expected to close more easily in order to enhance the use of the dominant eye, and as a corollary, the dominant eye would be more resistant to closure unilaterally because of the very nature of its dominance. In putting this theory to test, we used the students of our senior and junior anatomy classes (total 145, ranging from 18 to 24) as subjects. Eye dominance was first ascertained by three or more trials of the cone test in each case, and then the subjects were asked to close the right and left eyes singly and alternately for a number of times and to say which eye they found could close with less effort. This latter test was subjective; in some cases, however, we were able to see quite plainly for ourselves. The results of our investigation are given in Table 1.

Table 1

No. of subjects	Dominant eye	Ease in closing
63	*R*	*L*
31	*L*	*R*
29	*R*	*R*
16	*L*	*L*
4	*R*	*ND*
2	*L*	*ND*

L, Left eye; *R*, right eye; *ND*, no difference between the eyes.

The above results show that two-thirds of the subjects could close the non-dominant eye more easily than the dominant eye, while one-third the reverse. The value of chi-square obtained (χ^2 = 15·527 and P = 0·01) shows a significant correlation between eye dominance and the relative ease of closure of the non-dominant eye. In addition, the results show that the differential function of the orbicularis oculi, innervated by the facial nerve, is a component in the dynamic complex of eye dominance.

S. T. CHAN
K. S. F. CHANG

Department of Anatomy,
University of Hong Kong,
Hong Kong. Nov. 28.

Presence of Aldosterone-stimulating Hormone in Urine

The secretion of aldosterone differs from that of other steroids of the adrenal cortex in that it is not directly under pituitary control. Current concepts favour a location in the diencephalon for the centre which controls aldosterone secretion[1]. The transmission of the stimulus from the centre to the effector gland cannot be nervous, since the isolated gland can respond to suitable provocations by an increased secretion of aldosterone[2]. It is therefore concluded that the stimulus is humoral.

Evidence for the presence in the urine of adrenalectomized rats deprived of sodium of a substance which modifies the secretion of an electrolyte-active steroid when injected into intact rats has been presented by Orti *et al.*[3]. This substance may be the hypothetical hormone responsible for the stimulation of aldosterone synthesis or release. The present investigation confirms the presence of this substance in urine under certain conditions, and evidence is presented that it is an 'aldosterone-stimulating hormone'.

Rats of Wistar strain, weighing about 150 gm., were bilaterally adrenalectomized and maintained on rat cake, with 1 per cent sodium chloride solution to drink. Groups of 20 of these animals were deprived of food and drink for 36 hr., and their urine collected over the last 12 hr. of this period. This urine was injected subcutaneously into groups of 5 intact rats, together with a sodium load of 1 ml. of 0·45 per cent sodium chloride solution per 50 gm. body-weight, administered intraperitoneally. 5 intact rats served as controls, receiving a similar sodium load intraperitoneally and a volume of urine from adrenalectomized rats receiving 1 per cent saline was given subcutaneously, equal in volume to that of the urine injected into the experimental group. Urine was collected individually from the 10 rats at intervals of 40 and 80 min. after the injection of the urine. Cross-over tests were made three days later. Statistical analysis of variance shows that the administration of urine from the salt-deprived animals resulted in a lowering of the sodium/potassium ratio in the urine of the intact animals compared with the control group, as shown in Table 1.

Table 1

No. of rats	Time (min.)	Mean sodium/potassium, experimental	Mean sodium/potassium, control	*P*
40	40	1·71	2·34	0·005
40	80	1·09	1·37	0·01

The urine from the experimental and control groups was collected in separate pools. This urine was hydrolysed for 48 hr., extracted with chloroform and run on the chromatographic system of Zaffaroni *et al.*[4] (toluene–propyleneglycol), or the '*B*5' system of Bush[5]. Insufficient material was present for a definite chemical identification of aldosterone to be made. The material running in the position of aldosterone was eluted from paper and bio-assayed in adrenalectomized rats. The urine from the experimental group possessed sodium-retaining activity.

Serial assay on a similar pool of urine showed that the substance was stable at 4° C. for seven days. It would also withstand boiling for 5 min. with little loss of activity.

These observations substantiate the presence in the urine of dehydrated adrenalectomized rats of a substance which, when injected into intact rats, causes a lowering of urinary sodium/potassium ratio, and circumstantial evidence is presented that this is due to the release of aldosterone. Corticotrophin, 1 U.S.P. unit per rat, did not possess this activity. It is concluded that the substance responsible for this effect is 'aldosterone-stimulating hormone'.

E. J. Ross
E. K. McLean
G. R. Miller

Medical Unit,
University College Hospital Medical School,
London, W.C.1.
Sept. 25.

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Biological Effects of Aromatic Extracts from Urine of Schizophrenics

Wada and Gibson[1] have recently reported that aromatic material extracted from the urine of schizophrenic patients induces behavioural and electroencephalographic abnormalities when injected intracisternally and intraventricularly in cats and monkeys. They used the fraction extracted by eluting with phenol-saturated water the material adsorbed on activated charcoal, according to the method of McGeer *et al.*[2].

Similar observations were made in the course of a series of experiments including the study of biological effects produced in mice, rabbits and dogs by aromatic fractions extracted from the urines of normal and schizophrenic subjects. The extracts were obtained by Decker's method[3], which is based upon elution with a water–methanol–butanol–ammonia mixture of the material adsorbed on activated charcoal. Of the group of substances thus extracted only the methanol-soluble fraction was investigated. Previous paper chromatographic analysis[4] of this fraction has shown that a larger number of aromatic substances is present in the urine of schizophrenic subjects.

The biological studies of such extracts included the determination of (*a*) the acute toxicity following intraperitoneal injection in mice; (*b*) the chronic toxicity in mice with histological examination of the brain and other viscera; (*c*) the behavioural effects following intracerebral injections in the rabbit; (*d*) the effect on the electrical activity of the cerebral cortex following topical applications in rabbits and dogs.

Both the aromatic fraction extracted from the urine of schizophrenic subjects and that from urine of normals have toxic properties. However, the fraction from schizophrenic patients appears to possess a higher acute toxicity and produces at lower dose-levels, upon chronic administration, degenerative changes of nerve cells of brain-stem nuclei. Furthermore, the aromatic urinary fraction from schizophrenic subjects has definite convulsant properties but only when brought into direct contact with the central nervous system. Intracerebral injections of minute amounts of the diluted extracts are readily followed by prolonged and generalized convulsions.

Direct applications of the diluted material to very small areas (2 sq. mm.) of the surface of the cerebral cortex cause localized convulsive alterations of the electrocorticogram.

DARGUT KEMALI

Neuropsychiatric Institute,
University of Naples,
Policlinico, Naples.

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Proximo-Distal Movement along the Axon of Protein synthesized in the Perikaryon of Regenerating Neurons

DURING regeneration of crushed nerve-fibres of spinal ganglion cells, the turnover rate of proteins in the ganglia (*C*.7 and *C*.8) increases extensively, as compared with the controls[1]. This finding, on one hand, justifies the increase in protein in the perikaryon of the regenerating neuron[1,2]; on the other hand, it supports Weiss's opinion[3] that some of the new proteins leave the body-cell and reach the growing end of the axon.

Movement of the protein along the axon can be studied by tagged compounds[4,5], especially when a set experimental condition is followed; that is, *in vivo* only the perikaryon of regenerating nerve cells must synthesize labelled proteins from tagged precursors (for example, *C*14-amino-acids). Thus, if the 'new' proteins move along the axon, it should be possible to collect them at the regenerating end of the nerve fibre. It is clear that a labelled precursor of proteins in the blood would contaminate all boundaries of crushed neurons, giving rise to difficulties in the evaluation of the phenomenon.

Hypoglossal and vagus nerves of adult rabbits were exposed on one side and crushed by forceps cooled to −70° C.; the former at the level of the intermediate tendon of the digastric muscle; the latter at the carotid sinus. After 13 days, an opening was made on the middle nuchal line and a small window (~3 mm. diam.) was made through the atlo-occipital membrane. After removing the tela chorioidea of the fourth ventricle and drying the cerebrospinal fluid by filter paper, when necessary, the calamus scriptorius becomes clearly visible. About 0·001 ml. of Krebs and Henseleit solution[6], containing 0·2 gm. per cent of glucose, 5·55 mgm. per cent of L-threonine-^{14}C (*S.A.*, 32·9 μc./mgm.) and 7·8 mgm. per cent of L-valine-^{14}C (*S.A.*, 68·5 μc./mgm.), was placed on the calamus, using a micrometer syringe; the procedure was repeated every 20 min. for 5 hr. In this way it was possible to concentrate the tagged amino-acids in the region of the hypoglossal nucleus and dorsal nucleus of the vagus, without introducing significant quantities of carbon-14 compounds into the blood or cerebrospinal fluid.

At different intervals after the experiment, the growing segments of the XII and X nerves, as well as the homologous segments of the other side, used as controls, were removed. Each tissue sample was homogenized in 9 vol. of 5 per cent cold trichloracetic acid and the suspension centrifuged; the procedure was repeated twice and the residue was washed successively three times with acetone and three times with ether. Finally, the dry residue was hydrolysed in 1 ml. of 6 *N* hydrochloric acid in a sealed tube at 115° C. for 18 hr. The hydrolysate was brought to dryness at 50° C. in air stream, taken up in water and transferred to a planchet for determination of radioactivity in a gas-flow counter.

The results can be summarized as follows: (1) During the first 30 hr. after commencement of the experiment, the regenerating segments of the XII and X nerves showed no radioactivity. (2) In the next 10 hr., the regenerating segments displayed a significant amount of radioactivity. This radioactivity was inseparable from threonine and valine, as was demonstrated by autoradiograms of the amino-acids from the protein hydrolysate separated by paper chromatography. The control segments did not show any radioactivity. (3) Between the fortieth and seventy-second hour, radioactivity was still present within the regenerating segments; no radioactivity was found in the homologous normal segments.

A detailed account of the experiments will be published elsewhere.

N. MIANI

Department of Anatomy,
University of Padua.
Nov. 25.

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Hæmostatic Mechanisms in the Animal Arterial Wall

DEPOSITION of fibrin on the interior surfaces of arteries after intimal injury forms the basis of the thrombogenic theory of the pathogenesis of arteriosclerosis[1]. Such fibrin deposits, covered by endothelium, have repeatedly been observed[2–6].

Fibrin deposition in the organism is determined by a hæmostatic balance between fibrin formation and fibrin resolution, which is regulated by the mechanisms of blood coagulation and fibrinolysis[7,8]. In order to try to substantiate the role of fibrin deposition as a primary process in the development of arteriosclerotic lesions, we studied the thromboplastic and fibrinolytic activities of the separated layers of the wall of the human aorta. We found high thromboplastic activity in the central layers (intima and media) and less activity in the adventitia. Little or no fibrinolytic activity was present in intima or media, while adventitia was highly fibrinolytic[9]. These results were verified by additional studies comprising samples of aortæ with varying degrees of atheromatosis[10]. The high thromboplastic activity of the human aorta (including intima) was recently confirmed[11].

It has proved difficult, if not impossible, to produce experimentally in animals pathological changes identical with human arteriosclerotic lesions. This observed fact has never been satisfactorily explained.

However, an investigation of the fibrinolytic activity of samples of aortæ from monkeys demonstrated considerable activity in intima and media[10]. Furthermore, in bovine aorta low thromboplastic activity was found in the intima, while media and

adventitia had higher activity[12]. Hence, animal arteries apparently differ from human vessels in their hæmostatic systems.

In order to elucidate this problem we have recently compared the aortic walls in some animal species. The procedures were the same as applied in our previous studies[10]. Freshly drawn citrated rabbit plasma served as test substrate for the thromboplastic activity. The concentration of plasminogen activator in the tissue was estimated after extraction with 2 *M* potassium thiocyanate[13]. The results of this investigation are summarized in Table 1.

Table 1. Animal Species Arranged according to Hæmostatic System in Arterial Intima

Fibrinolytic activity in decreasing order; thromboplastic activity in increasing order. The results represent average values

Fibrinolytic activity

Animal	Adv.	M.		I.
Monkey	+ + +	+		+
Horse	+ + +	+		(+)
Dog	+ +	(+)		(+)
Rat	+ +		+ +	
Guinea pig	+		(+)	
Pig	+ + +	0		0
Ox	+	0		0
Rabbit	0	0		0

Thromboplastic activity

Animal	Adv.	M.		I.
Rat	Inhib.		Inhib.	
Dog	0	(+)		0
Guinea pig	+		(+)	
Monkey	+	+		(+)
Ox	+	+ + +		+
Pig	+ + +	+ +		+
Horse	+ +	+ + +		+ +
Rabbit	(0)	+ +		+ + +

Adv., adventitia; M., media; I., intima; Inhib., inhibition.

It is apparent that great variations in the hæmostatic systems in different animal species exist. It is interesting to note that the rabbit, a preferred animal for experiments on connective tissue and vascular lesions, is low in fibrinolytic activity and high in thromboplastic activity, indicating an increased tendency to form and retain deposits of fibrin. The rat, on the other hand, has a coagulation inhibiting mechanism in its arteries. In this connexion it is of interest to recall that heparin and mucopolysaccharides with anticoagulant activity have been isolated from arteries of some species[14–16]. Apparently regulation of fibrin deposition can be obtained by several means depending upon the species in question. It is also of interest that high fibrinolytic activity (in man) is predominantly related to the venous system[17].

In none of the species investigated does the hæmostatic system compare exactly with that in the human organism. Based on our previous studies[9,10] the fibrinolytic system in human arteries appears similar to that in the pig, while the thromboplastic system is more active than in the rabbit. This means that the tendency to form fibrin deposits is more pronounced in man than in the rabbit, at the same time as there is little ability to produce resolution by means of a local fibrinolytic activity in the tissue. This finding suggests that resolution of fibrin deposits in the arterial system in man depends primarily on the activity of the humoral system in the circulating blood, which in man is characterized by a higher latent activity than in most animals. On the basis of these observations we offer the suggestion that the lack of a suitable experimental animal in studies of human arteriosclerotic lesions is due to differences in the hæmostatic systems of their arteries and in the circulating blood.

The results of this work will be published in detail elsewhere. Grants were received from the Josiah Macy, Jr., Foundation, New York, and from the National Danish Association against Rheumatic Diseases.

Tage Astrup
Karol Buluk*

Biological Institute,
Carlsberg Foundation,
Copenhagen.
Nov. 10.

* World Health Organization Fellow. On leave of absence from the School of Medicine, University of Bialystok, Poland.

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Effect of Cations on γ-Aminobutyric Acid Level in Slices of Brain Cortex

Since the discovery by Roberts and Frankel[1] of the unusually high content of γ-aminobutyric acid in brain, other mammalian tissues containing only minute amounts, a number of reports have appeared concerning the significance of this amino-acid to the physiological activity of the central nervous system[2].

The present communication deals with the effect of the concentration of potassium and calcium ions in the incubation media on the content of γ-aminobutyric acid in the slices. It is not without interest to examine the dependence of the amount of physiologically active γ-aminobutyric acid on the concentration of the ions K^+ and Ca^{2+} in the medium, as the importance of environmental ionic composition for the nervous tissue metabolism is known[3,4].

Guinea pigs were used as experimental animals; in individual experiments six groups of slices from four animals were prepared and the amount of the amino-acid in the tissue plus incubating medium after incubation in various salines was compared. Slices were incubated aerobically at 37° for 4 hr. in normal and modified Krebs–Ringer saline, the incubation stopped by denaturing the proteins, and the free amino-acids present in the supernatant after centrifugation of the homogenized slices separated chromatographically in a mixture of *n*-butanol/formic acid/water (75 : 15 : 10). Practically the same results were obtained with a 2-hr. incubation. The estimation of γ-aminobutyric acid in the corresponding strips of the chromatogram was carried out according to Yemm and Cocking[5].

It was found that, as compared with the control, only the intensity of the γ-aminobutyric acid spot on the chromatogram was markedly changed on

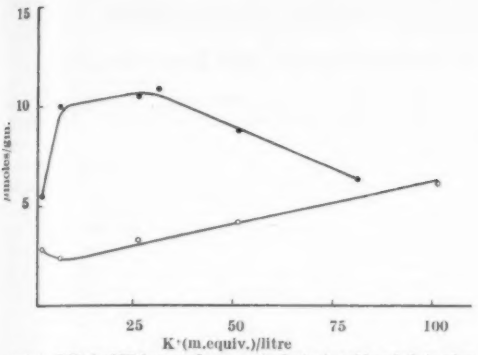

Fig. 1. Effect of K^+ ions on free γ-aminobutyric acid and glutamine level in slices of brain cortex. The amounts of γ-aminobutyric acid (empty circles) and glutamine (full circles), estimated in the incubation media plus saline, expressed in μmoles/gm. fresh weight

incubation in a medium containing 25 m.equiv. K^+/l. This observation was subjected to further analysis. As follows from Fig. 1, the γ-aminobutyric acid level is lowest during incubation in a medium containing 6·25 m.equiv. K^+/l. With raised K^+ concentration the amount of the amino-acid increases linearly and at 100 m.equiv. K^+/l. it reaches the value of 6·2 μmoles/gm. fresh tissue. Also, the lowered potassium concentration causes an increase in the amino-acid level. The average amount of the amino-acid present in fresh slices was 2·9 μmoles/gm.

It is well known that γ-aminobutyric acid is formed by decarboxylation of glutamic acid. In another set of experiments under the same experimental conditions changes in the content of a different amino-acid, also arising metabolically from glutamic acid —namely glutamine—were followed. The amide was estimated from the amount of ammonia released on hydrolysis with trichloracetic acid, as described by Harris[6]. The decrease in glutamine formation, representing an endergonic reaction, with increasing amount of potassium in the incubation media is not surprising, the impairment of energy metabolism by potassium concentrations having been already repeatedly reported[3]. It appears (cf. Fig. 1) that no direct correlation exists between the γ-aminobutyric acid and glutamine levels in the slices.

Elliott and Florey[7] pointed out that a fraction of γ-aminobutyric acid is found in the brain tissue in a thermolabile 'bound' form hitherto not exactly defined. The question arose whether the increase in the γ-aminobutyric acid content was not partly due to the release of the acid from this labile form; therefore the amount of γ-aminobutyric acid was compared in boiled and unboiled homogenates after incubation in various media. It was found in preliminary experiments that the amount of this amino-acid was higher in the boiled homogenates after incubation of slices in the media with 1·25 and 6·25 m.equiv. K^+/l. After incubation of brain tissue at higher concentrations of potassium the amount of the acid was nearly the same in boiled and in unboiled homogenates. It appears that the release of γ-aminobutyric acid from the thermolabile bond could be considered as a factor contributing to the increase in the level of the free amino-acid at higher concentrations of potassium in the saline. Furthermore, the possible activation of the glutamic acid decarboxylase under the experimental conditions described should also be taken into account.

On omitting calcium ions from the incubation medium the amount of γ-aminobutyric acid is increased, which is in agreement with the already well-established antagonistic action of calcium and potassium ions on brain cortex metabolism.

Details of this work will be published elsewhere.

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R. Rybová

Laboratory for Cellular Metabolism,
Biological Institute,
Czechoslovak Academy of Sciences,
Prague.

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Attempted Extraction of Crustacean Moulting Hormone from Isolated *Y*-Organs

Echalier[1,2] has shown, by extirpation and implantation experiments, that the *Y*-organs of Crustacea[3] produce the moulting hormone. The *Y*-organs are therefore physiologically (and morphologically) homologous to the prothoracic glands of insects. It has also been demonstrated[4,5] that extracts from whole Crustaea (*Crangon vulgaris*[6], *Emerita talpodia*, *Libinia* spp.[7]) are active in insects, initiating puparium formation in ligated *Calliphora* larvæ. Thus, they have the same action as ecdysone, the moulting hormone or prothoracic gland hormone of insects.

We have been interested in learning whether the arthropod moulting hormones are stored in the glands of internal secretion, or if they are produced there and then released into the blood. Excision of prothoracic glands of insects would be extremely difficult; in contrast, the anatomy of the crustacean *Y*-organ allows preparation of the glandular tissue for chemical investigations.

100 *Y*-organs were dissected from green crabs (*Carcinus maenas*) of about 75-mm. carapace width, in stage C_4 (that is, the intermoult period) of the moulting cycle, and preserved in methyl alcohol. 36 carcasses were also stored. The glands were then homogenized in methanol, centrifuged and re-extracted with methanol. The methanolic extracts were combined, evaporated to a small volume, diluted with water and extracted first with ethyl ether, then with butanol; the butanol solution was taken to dryness, yielding 12·5 mgm. extract.

The carcasses were homogenized in a Waring blendor and extracted with methanol. The methanolic extracts (approx. 3 l.) were combined, concentrated to a small volume, and, after dilution with water, extracted first with ether, then with butanol; the butanol solutions were washed with 0·2 *N* sulphuric acid, 2 *N* sodium carbonate, water, 1 *N* acetic acid and water, and evaporated to dryness. The residue (5·12 gm.) was dissolved in butanol, adsorbed on 50 gm. alumina (activity *V* according to Brockmann) and eluted with 200 ml. butanol (fract. 1), 200 ml.

Table 1

Starting material	Total final extract (mgm.)	Amount injected (μgm.)	Puparium formation (per cent)	*Calliphora* units (ref. 8)
100 *Y*-organs	12·5	250	0	<10
36 Carcasses without *Y*-organs	Fract. 1 127	50	58	2,500
	Fracts. 2–3 913	50	33	9,000

butanol (fract. 2) and 500 ml. butanol–methanol 1 : 1 (fract. 3). Fract. 1 (2·1 gm.) was dissolved in ether, then extracted with water, and the aqueous layer extracted with butanol. The yield, after evaporation of the solvent, was 127 mgm. Fracts. 2 and 3 were combined (1·15 gm.) and treated in the same way: yield, 913 mgm. The purified extracts were bioassayed in *Calliphora* larvæ (cf. ref. 8) (Table 1).

From the result of the bioassay, it is obvious that the *Y*-organs do not store measurable quantities of the hormone: 100 glands contain less than 10 *Calliphora* units, while 36 carcasses contain more than 10,000 units. These results were obtained using intermoult crabs; it remains to be seen whether the same relation holds for pre-moult crabs, and for insects. In the latter case, however, removal of prothoracic glands is not feasible because of their diffuse nature.

The positive response of *Calliphora* to *Carcinus* extracts demonstrates once more the interchangeability of insect and crustacean moulting hormones. There is little doubt that the *Calliphora* assay is reliable in this respect, though confirmation by assay in other insects, for example, *Rhodnius*[9] or *Cecropia*[10], might be desirable. The chemical properties of the crustacean hormone differ from the insect hormone: the former is more strongly adsorbed to alumina.

Another insect gland, the corpus allatum, which produces the juvenile hormone, contains undetectable amounts of active material. The juvenile hormone is either rapidly destroyed[11] or excreted[12] or piled up in the abdominal tissue[13]. Apparently, the endocrine glands of arthropods do not function as storage organs.

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PETER KARLSON

Physiol. chem. Institut d. Universität,
München,
Germany.

DOROTHY M. SKINNER

Graduate Department of Biochemistry,
Brandeis University,
Waltham, Mass.

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PLANT PHYSIOLOGY

Gravimorphism and Root Formation

IT has previously been reported[1,2] that gravity can affect the growth and flowering of trees. The results to be described indicate that gravity may also affect the growth of some herbaceous plants in that the orientation of the shoot can determine whether or not root initials are formed in the stem.

Pot-grown plants of *Bryophyllum daigremontianum* (R. Hamet et Perv.) Berg. and tomato var. 'Moneymaker' were placed so that the shoots and the pots were horizontal. Staking ensured that the shoots remained horizontal within practical limits. One group of horizontal plants had the same side uppermost throughout, while a second group was placed on a horizontal klinostat which rotated the plants approximately once an hour. Preliminary experiments showed that rotating the plants through 180° once or twice each day did not prevent the formation of adventitious roots. A third group of plants was maintained with the stems and pots vertical. To facilitate watering, all the horizontal plants were placed erect for a short period each day.

After a few weeks of such treatments, adventitious roots were clearly visible on the underside of the stem internodes of those plants that were maintained with the same side uppermost. In *B. daigremontianum* these adventitious roots were visible after 2–3 weeks and continued to increase in size and number during the ensuing weeks (Fig. 1). At the end of the experiment many of the roots exceeded 2·0 cm. in length. Root formation in tomato was slower, taking 4–5 weeks to become visible. Under the conditions of the experiment tomato roots did not extend, although the lower side of the stems became covered with numerous root primordia. In both species roots were confined to the lower side of the horizontal stems. The rotated and vertical plants did not exhibit any abnormal propensity to form roots. Table 1 summarizes the results of one experiment with *B. daigremontianum*. The undersides of the horizontally placed stems were shaded, and it is not known how much this differential illumination may have affected the results[3,4]. This will be investigated and reported on in due course.

Fig. 1. Adventitious roots arising from a horizontally placed stem of *Bryophyllum daigremontianum*

Table 1. The Effect of Gravity on the Adventitious Root Formation of *Bryophyllum daigremontianum*

Stem position	Duration of experiment	No. of plants	No. of visible adventitious roots
Horizontal, rotated	37 days	6	2
Horizontal, non-rotated		6	72
Vertical		6	0

Loeb[5] observed that ". . . in *Bryophyllum calycinum* the substances which induce root formation have a tendency to collect on the lower side of a horizontally placed stem although roots may also appear in the nodes on the upper side. . . ." All the roots in Loeb's drawing were of nodal origin and not predominately internodal as in the present experiments. Resende[6], working with *B. daigremontianum*, noted that roots formed on the stems of horizontally placed plants at the point where the stems curved as a result of geotropic action. Both authors appear to have been recording manifestations of gravimorphism similar to those already described.

These observations are of horticultural significance in relation to the practice of layering used for the propagation of difficult subjects. Further, the rapid response of *B. daigremontianum* presents a ready means of demonstrating one of the effects of gravimorphism to students.

A more detailed account of this work will be published elsewhere.

J. K. A. Bleasdale

National Vegetable Research Station,
Wellesbourne,
Warwick.

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Fungitoxicity of Metal Ions

In a recent communication, Somers[1] has again considered the hypothesis first proposed by Mathews[2], that the toxicity of metal ions is related to the electrode potential of the metal. He has plotted the negative logarithms of the *ED*50 values of the applied molar concentrations of 21 elements for conidia of *Alternaria tenuis* against the electronegativity of the elements. An examination of the curve indicates clearly a correlation between the electronegativity and toxicity in the data presented. This is especially fortified by the low electronegativity of magnesium, strontium, lithium, sodium, barium and potassium ions which are basically not toxic to fungus conidia. If, however, attention is confined to that part of the curve which deals with ions toxic to fungus conidia (ions as toxic and more toxic than zinc), it is apparent that in a number of instances, the *ED*50 values are several orders of magnitude higher or lower than if they fell on the line drawn. As a matter of fact, examination of the data for these 15 elements, by calculating the rank correlation coefficient (calculated by Dr. F. Wilcoxon, Boyce Thompson Institute), shows that there is no significant correlation between electronegativity and toxicity in the area in which the toxic elements are found.

It should be pointed out that much information has been accumulated in recent years showing that the concentration of the applied solution is a poor measure of toxicity[3–9]. Fungus conidia have a great affinity for toxic ions and compounds as a result of which they often will take up lethal doses from dilute solutions in a minute or less. The applied concentration is thus maintained for a short time only and is important, not as such, but because it determines the dose available per unit of fungus conidia. Two toxicants may give equal *LD*50 values on an applied concentration basis but yet differ a hundredfold or more in innate toxicity. This follows because the germination response is determined by the quantity of toxicant taken up by the conidia with little regard to how such uptake was brought about. This is true for most of the toxicants studied, although there are exceptions. With zinc and cadmium the toxic effects are influenced by the length of exposure to the toxicant solution even when similar amounts are taken up[7].

Obviously the amount of a given ion taken up by conidia depends upon the quantities of binding sites in the spores and their availability to the ions presented in the ambient solution. The resultant toxicity would be determined by the essential nature of the binding sites occupied, and reversibility would be possible if the bond between the toxicant and the binding sites can be broken without impairment of essential functions. It is apparent, as also brought out by Somers[1], that chemical reactions occur between the applied ions and cell constituents. Such reactions can occur after the conidia are no longer capable of germination and thus can account for uptake of toxicants by spores no longer viable[4].

The question arises as to where these reactions between binding sites and the applied ions occur. Somers[1], in common with some others, believes that they occur at the cell surface. This point of view results from a belief that the conidia are permeable with difficulty to ions and compounds supplied and, moreover, it assumes that enough of the essential processes of the cell takes place at the surface, so that reaction at the surface can destroy germinative capacity. Experiments conducted at the Boyce Thompson Institute over the past ten years with labelled ions and compounds and labelled cell constituents have shown that a much freer movement of materials takes place across the membranes involved than was previously supposed. If fungus spores are collected without immersion in water and are then suspended in water, from 10 to more than 30 per cent of the cell constituents is released into the ambient solution[10]. The remaining contents are more firmly held, but further release will take place, without impairment of germination, on exposure to low doses of certain potentially toxic agents such as silver. Silver has been found to affect the conidia in such a way as to bring about a release of phosphorus compounds, as determined with spores labelled with phosphorus-32[6,7]. Effects of silver on permeability are also shown by its action in increasing the uptake of other ions supplied, such as mercury and cerium[8].

Sonic disintegration of spores previously treated with relatively low doses of silver, mercury, cadmium, zinc, or cerium, and determination of the distribution of the labelled ions in various fractions isolated have not indicated an accumulation of these elements at the cell surface[11]. While there is a possibility of some redistribution taking place during the disintegration process, experiments indicate that very little redistribution actually occurs.

When conidia of different species are compared the quantity of toxicant that can be taken up is found to vary greatly from species to species. A species taking up very large amounts of one toxicant will not necessarily take up comparatively large amounts of a second toxicant. Also the ion may not be very toxic to a species which has a great affinity for it.

Experiments being carried out with cerium have shown that with some species the binding sites for cerium are readily available to cerium supplied in the ambient solution. With others the addition of 'Tergitol-7' (sodium heptadecyl sulphate) or CN^-, in non-toxic quantities, makes available further binding sites and thereby increases cerium uptake. The total potential cerium uptake, of course, also varies greatly among the species studied.

Ion interference studies now being conducted indicate that the interaction between conidia and the metal ions is much more complex than can be explained by differences in electronegativity. Thus cerium would not be expected to compete with silver or mercury for the same receptor sites, yet small amounts of cerium seriously interfere with the uptake of silver and mercury.

The evidence strongly indicates that much more than cell surface reactions is involved in the toxicity of metal ions to conidia.

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Lawrence P. Miller

Boyce Thompson Institute for Plant Research, Inc.,
Yonkers 3, New York.
Nov. 12.

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Hydrogenation of Chloroplast Lipids by Rumen Bacteria

It was reported in an earlier paper[1] that rumen protozoa can hydrogenate unsaturated lipids. The suggestion was made that rumen bacteria may not contribute a great deal to hydrogenation of dietary lipids ingested by ruminants. This latter conclusion was based on experiments using washed suspensions of rumen bacteria, incubated anaerobically in phosphate buffer containing sodium sulphide, with either linseed oil, or linoleic or linolenic acids. Formic acid, glucose or finely divided plant material were also added in these experiments. Estimation of the iodine numbers of the total fatty acids in the samples showed either no change or only a slight decrease in the iodine values.

Since these results were considered rather unexpected, the role of the bacteria in hydrogenation has been reappraised using chloroplasts as a source of unsaturated lipid substrate. Rumen liquor was obtained from a rumen-fistulated cow routinely fed on fresh red clover (*Trifolium pratense*), care being taken to maintain both the temperature of the sample and anaerobic conditions. Washed suspensions of the bacteria were prepared by a method similar to that described by Doetsch, Shaw, McNeill and Jurtshuk[2]. The suspension was divided into two equal portions, one of which was placed in hot water (90°) for 30 min. to destroy enzyme activity. Equal aliquots of a chloroplast suspension[1] together with glucose (0·25 gm./100 ml.) were added to both samples and the flasks incubated overnight at 39° under carbon dioxide.

A second similar experiment was carried out using strained rumen liquor from which the protozoa and plant material had been removed by centrifugation (1,000*g* for 5 min.). Chloroplasts and glucose were added to the suspensions, one of which had been heated as above, and the flasks incubated overnight at 39° under carbon dioxide.

The samples were freeze-dried and the lipids extracted by boiling successively with acetone, absolute ethanol and diethyl ether. The solutions were evaporated to dryness, taken up in petroleum ether and the solvent removed *in vacuo*. The fatty acids from the lipids were isolated by the usual procedures[1] and their methyl esters analysed by gas-liquid chromatography.

Table 1. Carbon-18 Fatty Acid Composition of Chloroplast Lipids incubated with Rumen Bacteria

Sample + chloroplasts + glucose (0·25 per cent w/v)	Suspending medium	Fatty acids (moles per cent)			
		Saturated	Unsaturated		
			mono-	di-	tri-ene
Live bacteria	*M*/15 phosphate buffer (*p*H 7·0) + 0·02 per cent $Na_2S.9H_2O$	4·1	11·0	7·6	77·3
Heated bacteria		4·0	4·0	10·0	82·0
	Difference	+0·1	+7·0	−2·4	−4·7
Live bacteria	Rumen liquor *p*H 7·0	9·4	24·1	62·2	4·3
Heated bacteria		5·0	6·5	5·0	83·5
	Difference	+4·4	+17·6	+57·2	−79·2

The results (Table 1) show a great contrast in hydrogenation activity between the bacteria suspended in phosphate buffer and those in rumen liquor. The bacteria incubated in phosphate buffer have caused only a slight, although definite, hydrogenation of the unsaturated fatty acids contained in the chloroplasts. There has been some conversion of triene to diene and monoene acids in the live bacteria sample. The bacteria suspended in rumen liquor have converted nearly all the triene acid to more saturated acids, particularly the diene acids. It is interesting that there is a build-up of the diene acids as this corresponds to the results found with rumen protozoa when it was suggested that the enzymes responsible for hydrogenation showed some specificity and attacked the triene acids more than the diene acids.

The low degree of hydrogenation in the washed suspensions is presumably the result of either harmful effects caused in the handling and preparing of these

suspensions or the presence of some factor in rumen fluid that facilitates hydrogenation and explains the previous conclusions regarding the role of bacteria in ruminant hydrogenation. However, these results show there can be no doubt that rumen bacteria are capable of extensive hydrogenation, and emphasize the caution needed in the interpretation of results obtained under conditions far removed from those found *in vivo*.

I wish to thank Miss J. Michael for her able assistance and the Fats Research Laboratory, Wellington, for the gas–liquid chromatography analyses.

D. E. Wright

Plant Chemistry Division,
Department of Scientific and Industrial Research,
Palmerston North, New Zealand.

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HÆMATOLOGY

Red Cell Agglutination by Stromal Material in Hæmolysates

During the preparation of hæmoglobin solutions a phenomenon was observed which, so far as is known, has not previously been described. The red cells of some species of animals are agglutinated by hæmolysates made from the cells of the same species. The degree of agglutination is sufficiently strong to be easily visible to the naked eye when tested on a slide.

Hæmolysates were prepared from the cells of a number of different animals by subjecting a 20 per cent suspension of washed cells to ultrasonic vibration. After centrifuging for 20 min. at 3,000 rev./min. to remove the larger particles of stroma, it was tested against washed cells of the various species. The results are shown in Table 1, where the degree of agglutination is recorded, following the usual practice of blood transfusion work[1]. From Table 1 it can be seen that the agglutination seems to be a property of the hæmolysate rather than of the intact cell; and that it is confined to the three rodent species.

Table 1

Cells of	Hæmolysates of cells of: Sheep	Rabbit	Dog	Horse	Rat	Guinea pig
Sheep	0	+	0	0	0	+
Rabbit	0	+ + +	0	0	+ + + +	+ +
Dog	0	0	0	0	+	+ +
Horse	0	+ + + +	0	0	0	0
Rat	0	+	0	0	+ + +	+
Guinea pig	0	0	0	0	+	+

Group *A* and *O* human cells were not agglutinated by any hæmolysate and hæmolysates of human cells of groups *A* and *O* caused no agglutination of cells of any species.

If packed washed rabbit cells are frozen and thawed with the minimum of disturbance a jelly-like material with the consistency of egg-white is produced. This material, which is probably stromatin[2], can be filtered off and washed. The filtered hæmoglobin, although very concentrated, produces no agglutination of intact red cells. On the other hand, the washed residue, although not entirely free of hæmoglobin, is very active in producing agglutination when dissolved in saline. This property can be removed by absorption with rabbit cells but is unaffected by treatment with fat solvents.

The properties of the hæmolysates are dependent on the method of preparation. Fresh washed rabbit cells were lysed: (1) by ultrasonic vibration; (2) by the addition of distilled water followed by the calculated quantity of 10 per cent saline to make the solution isotonic and (3) by freezing. The hæmolysates were handled with care to avoid shaking. On completion of lysis all three preparations produced a similar degree of agglutination. After centrifuging at 96,000*g* for 1 hr. it was found that the supernatant fluids varied considerably in their ability to agglutinate washed cells. The deposit was re-suspended in saline by shaking vigorously for at least 5 min. before centrifuging at 3,000 rev./min. for ½ hr. The results of testing the supernatant fluid and the deposit against whole cells are summarized in Table 2.

Table 2

Method of production of hæmolysate	Hæmolysate: Deposit	Supernatant
1. Ultrasonic vibration	+	+ + +
2. Distilled water	+ + +	+
3. Freezing	+ + +	0

The probable explanation of these findings is that the more violent method of disruption of the cells also disintegrates the red cell envelope and stroma, some product of which is added to the solution and not removed by high-speed centrifugation for 1 hr. The gentler methods, however, leave this substance attached to the 'cell ghosts' which are found in the deposit.

We can conclude, therefore, that the agglutination of homologous and some heterologous red cells by hæmolysates of rabbit, rat and guinea pig red cells is due to a substance liberated from the stroma. The amount of this substance in the solution is determined by the method of lysis and the amount of shaking during preparation. This variation in composition may explain the divergent results obtained by the earlier workers in experimental hæmoglobinuric nephrosis.

S. B. de C. Baker

Department of Pathology,
University of Manchester.

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Asymmetrical Recombination of Alkali-dissociated Hæmoglobin Mixtures

The human adult hæmoglobins and canine hæmoglobin recombine asymmetrically, that is:

$$\alpha_2{}^X\beta_2{}^X + \alpha_2{}^Y\beta_2{}^Y = \alpha_2{}^X\beta_2{}^Y + \alpha_2{}^Y\beta_2{}^X$$

after both short and prolonged exposure to dilute acid[1–5]. At *p*H 11, hæmoglobin *A* dissociates into half-molecules[6]; furthermore, re-association of a mixture of labelled hæmoglobin *S* and hæmoglobin *A* after 24 hr. at *p*H 11 results in the exchange of the β_2 sub-units[7]. We now wish to report a series of experiments which indicate that hæmoglobin also recombines asymmetrically after short exposure to alkali.

In the first experiment a 2 per cent carbonmonoxy-hæmoglobin solution (all carbonmonoxyhæmoglobin

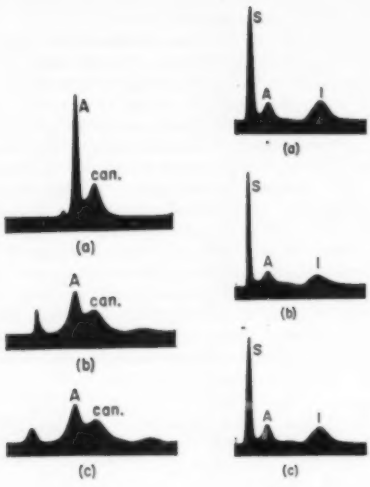

Fig. 1

Fig. 2

Fig. 1. Ascending-limb electrophoretic patterns of 1 per cent carbomonoxyhæmoglobin in phosphate buffer of *p*H 6·8, Γ/2 0·02. The direction of migration is from right to left. (*a*) *A*, canine control; (*b*) *A*, canine recombined after 20 min. dissociation at *p*H 11·2; (*c*) *A*, canine recombined after 20 min. dissociation at *p*H 4·7

Fig. 2. Ascending-limb electrophoretic patterns of 1 per cent. carbomonoxyhæmoglobin in phosphate buffer of *p*H 6·3, Γ/2 0·02. The direction of migration is from right to left. (*a*) *S*,*A*,*I*, control; (*b*) *S*,*A*,*I*, recombined after 20 min. dissociation at *p*H 11·2; (*c*) *S*,*A*,*I*, recombined after 20 min. dissociation at *p*H 4·7. Note the new component between *A* and *I* in (*b*) and (*c*)

solutions were electrodialysed prior to use), 1 per cent in canine hæmoglobin and 1 per cent in human hæmoglobin, was diluted to 1 per cent by adding an equal volume of a glycine–sodium hydroxide buffer of *p*H 11·0, Γ/2 0·2. The solution was dialysed against this same buffer at Γ/2 0·1 for 24 hr. at 5° C. followed by dialysis against three changes of potassium phosphate buffer of *p*H 6·8, Γ/2 0·02 for approximately 17 hr. Upon electrophoresis in the same phosphate buffer two new components appeared, one migrating more slowly than canine and the other migrating more rapidly than *A*, as was the case after exposure to dilute acid (Fig. 1). 20-min. exposure to the same glycine–sodium hydroxide buffer produced only very small amounts of the new components. It was found, however, that the addition of the hæmoglobin mixture to the glycine–sodium hydroxide buffer changed the *p*H to 10·5 and therefore further experiments were carried out with glycine–sodium hydroxide buffers of a higher *p*H. A buffer of *p*H 11·5, Γ/2 0·2, when added to an equal volume of 2 per cent carbonmonoxyhæmoglobin, resulted in a *p*H of 11·0, and a buffer of 11·8 resulted in a final mixture *p*H of 11·2. After 20-min. exposure, the amounts of the new components observed upon electrophoresis at *p*H 6·8 were found to increase with increase in the dissociation *p*H. In one experiment in which the *p*H 11·8 buffer was used, immediate neutralization to *p*H 6·9 was effected after 20 min. by the addition of sodium dihydrogen phosphate. In all the others, 20-min. exposure to alkali was followed by dialysis in the potassium phosphate buffer *p*H 6·8. These results are shown in Table 1 and Fig. 1. Human and canine carbonmonoxyhæmoglobin, when dissociated separately at *p*H 11·0 for 20 min. and re-

Table 1. Composition of Canine and Human Hæmoglobin Mixtures before and after Recombination

Dissociation (*p*H)	Dissociation (time)	Electrophoretic composition (per cent of total)*			
		New slow	Canine	*A*	New fast
Control	—	—	37	63†	—
10·5	20 min.	2–3	39	55	4
11·0	20 min.	5	37	51	7
11·2	20 min.	11	35	41	13
11·2	20 min.‡	12	36	42	10
11·0	24 hr.	25	26	20	29
4·7	20 min.	10	36	44	10
4·7	22 hr.	31	24	21	24

* All samples contained 50 per cent each of human and canine hæmoglobin before recombination.
† Includes 2 per cent A_2.
‡ Followed by rapid neutralization to *p*H 6·9 by addition of sodium dihydrogen phosphate.

associated, showed no change in electrophoretic composition. Dissociation and recombination did not alter the absorption spectrum (450–700 mμ) of any of the samples.

A 50–50 mixture of carbonmonoxyhæmoglobins *A* and *I* (the sample contained 40 per cent *A* and 60 per cent *I*) and carbonmonoxyhæmoglobin *S* was dissociated at *p*H 11·2 for 20 min., neutralized rapidly to *p*H 6·9 by the addition of sodium dihydrogen phosphate and dialysed against three changes of potassium phosphate buffer of *p*H 6·3, Γ/2 0·02 for about 18 hr. Moving-boundary electrophoresis in the same buffer showed a new component migrating between *A* and *I*, a decrease in the proportions of *S* and *I* and increase in the proportion of *A*. The dissociation and recombination of *A* and *I* alone produced no change in the electrophoretic pattern. The results are given in Table 2 and Fig. 2.

Table 2. Composition of *A*, *I* and *S* Hæmoglobin Mixtures before and after Recombination

Mixture	Dissociation (*p*H)	Dissociation (time)	Electrophoretic composition (per cent of total)			
			I	New	*A*	*S*
A + *I*	Control	—	54	—	46	—
A + *I*	11·2	20 min.	56	—	44	—
A + *I* + *S**	Control	—	30	—	17†	53
A + *I* + *S*	11·2	20 min.‡	25	7	18	50
A + *I* + *S*	4·7	20 min.§	29	4	18	49

* All *A* + *I* + *S* samples contained 50 per cent hæmoglobin *S* and 50 per cent *A* – *I* hæmoglobin mixture, the latter containing 40 per cent *A* and 60 per cent *I*.
† Includes 1 per cent of trailing shoulder.
‡ Followed by rapid neutralization to *p*H 6·9 by addition of sodium dihydrogen phosphate.
§ Followed by rapid neutralization to *p*H 6·4 by addition of disodium hydrogen phosphate.

An attempt to perform similar experiments with human hæmoglobin *A* using the 'hæm-labelling method'[2] was unsuccessful, due to the rapid denaturation of methæmoglobin at *p*H 11 and above.

The appearance of two new electrophoretic components after alkalinization and neutralization of a mixture of human hæmoglobin *A* and canine hæmoglobin parallels the findings after exposure to dilute acid. The net change can be represented by the equation:

$$\alpha_2^A\beta_2^A + \alpha_2^{can}\beta_2^{can} = \alpha_2^{can}\beta_2^A + \alpha_2^A\beta_2^{can}$$

Similarly, the appearance of a new component between *A* and *I* and an increase in the proportion of *A* after alkali dissociation and neutral re-association of a mixture of hæmoglobins *A* and *I* and hæmoglobin *S* parallels the findings after acidification. The net change in this case can be represented by the equation:

$$[\alpha_2^I\beta_2^A]^{-1} + [\alpha_2^A\beta_2^S]^{+1} = [\alpha_2^I\beta_2^S]^{-1} + [\alpha_2^A\beta_2^A]^0$$

It would appear that the mode of recombination of a mixture of hæmoglobins after exposure to dilute alkali is the same as that following exposure to dilute acid, that is, asymmetric.

Although at equilibrium carbonmonoxyhæmoglobin *A* is completely dissociated at *p*H 11 and but partially dissociated at *p*H 4·7 (ref. 6), our results show that the amount of asymmetric recombination is the same after 20 min. at either *p*H. Complete recombination occurs in either case after 22–24 hr. The extent to which partial recombination occurs after a short period of dissociation thus appears to reflect more closely the rate of exchange of sub-units rather than the equilibrium degree of dissociation.

Acidification of hæmoglobin results in asymmetric dissociation into α_2 and β_2 sub-units[2]. Asymmetric dissociation in alkali would provide the simplest mechanism compatible with our present results. If, as proposed by Vinograd and Hutchinson[8], hæmoglobin dissociates symmetrically into the half-molecule $\alpha\beta$ in alkali, the mechanism of recombination in alkali must be more complex than in acid.

We are indebted to Dr. L. M. Tocantins for the sample of *A–I* hæmoglobin.

ELIZABETH ROBINSON
HARVEY A. ITANO
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland. Nov. 6.

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PATHOLOGY

Plasma Erythropoietin in Polycythæmia Secondary to Renal Disease

AN increasing body of experimental work indicates an intimate relationship between the kidney and the plasma erythropoietin-level[1–4]. It has long been recognized that in man there is a frequent association between chronic renal disease and anæmia, and it has been demonstrated that the plasma erythropoietin-level is reduced in chronic renal failure[5]. More recently, the association of certain other renal pathological conditions (for example, carcinoma of the kidney and hydronephrosis) with polycythæmia has been recognized[6,7]. However, studies on the plasma erythropoietin-level do not appear to have been reported in polycythæmia secondary to renal disease.

This communication is a preliminary report of studies on two such cases (to be reported more fully at a later date).

The two patients were middle-aged men in whom the diagnosis of polycythæmia was confirmed by red cell volume studies using chromium-51. The renal lesions were unilateral hydronephorosis in case I and a unilateral polycystic kidney in case II. In both cases, nephrectomy was performed, and the studies were repeated approximately four months after operation, at which time they were no longer polycythæmic.

Table 1

		Hb (gm. per cent)	P.C.V. (per cent)	Reticulocytosis in rats 72 hr. after injection of plasma (per cent)
Case I	Preoperative	18·3	55·0	5·7 6·1 Mean = 5·9
	16 weeks after operation	14·6	44·5	3·4 3·3 Mean = 3·4
Case II	Preoperative	18·7	55·0	8·9 8·4 Mean = 8·7
	19 weeks after operation	15·2	47·5	3·0 3·3 Mean = 3·2
Pooled plasma from five normal subjects		—	—	3·3 3·2 Mean = 3·3

Plasma samples were obtained pre-operatively and approximately four months post-operatively. The erythropoietin content of these samples was assessed by measuring the reticulocytosis induced, in black hooded rats, 72 hr. after a 2 ml. intraperitoneal injection of plasma. The results are given in Table 1.

These two cases appear to demonstrate convincingly the association of certain renal lesions with polycythæmia and increased plasma erythropoietin-levels, and show that a return to the normal erythropoietic state follows removal of the pathological kidney.

We wish to express our thanks to Dr. J. L. Pinniger for helpful advice and for permission to publish this communication.

R. W. PAYNE
N. F. JONES
R. D. HYDE
Louis Jenner Laboratory, St. Thomas's Hospital, London, S.E.1.

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Identification of a Bovine Mucosal-Disease Virus isolated in Sweden as *Myxovirus parainfluenzae 3*

'BOVINE mucosal disease' is the collective name proposed for different syndromes characterized mainly by inflammatory changes in the mucosa of the gastro-intestinal or respiratory tracts, or both, of cattle. The ætiology was uncertain in spite of successful transmission experiments. By the use of tissue culture for virus isolation, what are apparently different viruses have been found associated with such syndromes, but only one of these, the virus of infectious bovine rhinotracheitis, has hitherto been investigated thoroughly.

Quite recently, Reisinger *et al.*[1] in the United States reported the isolation of a myxovirus from cases of

shipping fever, a respiratory disease in cattle. Abinanti and Huebner[2] found this virus, called *SF*–4, to be serologically identical to the prototype strain of *Myxovirus para-influenzae* 3 (ref. 3), called *HA*–1. which was first isolated by Chanock *et al.*[4] from cases of respiratory disease in children.

A contagious form of enteritis in cattle, which seemed to be a variant of the mucosal disease type, has been described from Sweden[5]. The clinical signs have varied from outbreak to outbreak; the gastro-intestinal signs may dominate in one outbreak and respiratory signs in another. Differences in clinical signs were also observed within herds and during an outbreak[6].

Bakos *et al.*[7] investigated cases of a bovine mucosal-type disease with mainly respiratory symptoms and a severe course at Umeå in northern Sweden during 1958–59. The disease seemed to be introduced by cattle purchased from other parts of the country. The first signs of the disease were serous conjunctivitis and rhinitis, often combined with excessive salivation, high fever and, in some animals, diarrhœa. Then the nasal discharge became muco-purulent, the salivation more intensive and buccal erosions sometimes appeared; coughing was common. In some instances watery diarrhœa was the only sign encountered. The malignant course led in one of the infected herds of losses of up to 70 per cent of animals by inanition and dehydration. A cytopathogenic agent was isolated in calf kidney tissue cultures from nasal and tracheal exudates of the animals in two of the nine herds investigated. This agent was neutralized by convalescent sera from animals in these herds. It passed 'Gradocol' membranes with an average pore diameter of 240 mμ but not 130 mμ. In calves free of neutralizing serum antibodies, the agent produced in experimental infection a mild form of mucosal disease of the same type seen on the farms.

At titration of this so-called Umeå-strain of virus in calf kidney tissue cultures, it showed only slight cytopathogenicity but was more cytopathogenic when inoculated at higher dilutions. Therefore the subsequent passages were carried out in two lines, one with undiluted inocula and the other diluted 10^{-2} before inoculation. The former has been carried for 23 passages and the latter for 33 passages. The viruses have been designated strain 23 and 33 respectively. Both strains showed hæmagglutinins against guinea pig and chicken erythrocytes and strong hæmadsorption of guinea pig erythrocytes to the tissues in the infected tubes. Of the strains 23 and 33, only the latter produced clear plaques.

The strains 23, 33 and Chanock's[4] strain *HA*–1 of para-influenza 3 virus[3] have been compared by titration in calf and monkey kidney tissue cultures using lactalbumin-Hanks' solution (0·5/99·5 per cent) as maintenance medium. The hæmadsorption and hæmadsorption-inhibition tests were performed according to Chanock's modification[4] of the method described by Shelokov *et al.*[8], and the hæmagglutination, hæmagglutination-inhibition, and neutralization tests according to the standard techniques. In *HI* tests, the Umeå serum has been used untreated, and treated with *RDE* (cholera filtrate, Philips-Roxane, Holland). No difference could be observed in the antibody titres between the treated and untreated samples. In some of the experiments the *HA*-2 strain[4] of para-influenza 1 virus[3] and a serum against this strain were used for comparison.

The strain 23 showed slight or questionable cytopathogenicity for calf kidney cells only, and lower titres of infectivity for calf and monkey kidney cells than the strains 33 and *HA*–1. The strain 33 was definitely cytopathogenic for calf kidney cells and the strain *HA*–1 for monkey kidney cells, both showing approximately the same titres of infectivity. In neutralization tests monkey kidney cells were used for the strain *HA* 1 and calf kidney cells for the strain 33.

The results of cross-neutralization tests, summarized in Table 1, indicate a close relationship between the Umeå virus and the *HA* 1 strain of para-influenza 3 virus.

Table 1. Evidence for the Serological Relationship between the Virus Umeå and the Strain *HA* 1 of Para-influenza 3 Virus

Serum	Hæmadsorption inhibition[a]				Hæmagglutination inhibition[b]			Neutralization[c]	
	23	33	*HA* 1	*HA* 2	23	33	*HA* 1	33	*HA* 1
Umeå[d]	+	+	+	−	4096[e]	2048	1024	128[e]	512
HA 1	+	+	+	−	1024	1024	1024	256	1024
HA 2	−	−	−	+	<16	<16	<16	ND	ND

[a] Infected monkey or calf kidney tissue cultures;
[b] Against 4 hæmagglutinating units of each virus;
[c] Against 1,000 and 700 TCD_{50} of 33 and *HA* 1, respectively;
[d] Convalescent serum from a calf (No. 1) experimentally infected with the virus Umeå and with no antibodies before infection;
[e] Reciprocal of serum dilution:
ND, not done.

We are indebted to Dr. L. Philipson, of the Institute of Virology, Uppsala, for supplying the reference viruses and sera *HA* 1 and *HA* 2.

K. Bakos
Z. Dinter

State Veterinary Institute, Stockholm;
and Institute of Virology, University,
Uppsala.

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HISTOLOGY

Preparation of Ultra-Thin Sections of Mineralized Human Enamel

The preparation of ultra-thin sections of hard substances for electron microscopy without preliminary softening presents special difficulties. The use of industrial diamonds fashioned into knives suitable for cutting hard materials was first described by Fernández-Morán[1]. These knives, prepared with a cutting bevel of approximately 50–52°, can be used to cut or cleave thin sections of bone or tooth dentine. The sectioning of hard brittle materials, however, increases the risk of damaging the knife edge from glancing blows, since diamond is itself extremely brittle. The smallest natural cleavage angle obtainable from a diamond depends on the angles formed by the natural crystal planes (this angle is in the region of 70°), consequently in order to construct a diamond knife with the greatest possible edge strength, the two faces of the knife should form this angle. Two diamond knives both with the cutting faces diverging from the knife edge at an angle of 70° were prepared for us several years ago in the Johannesburg research laboratory of the Industrial Distributors, Ltd., under the

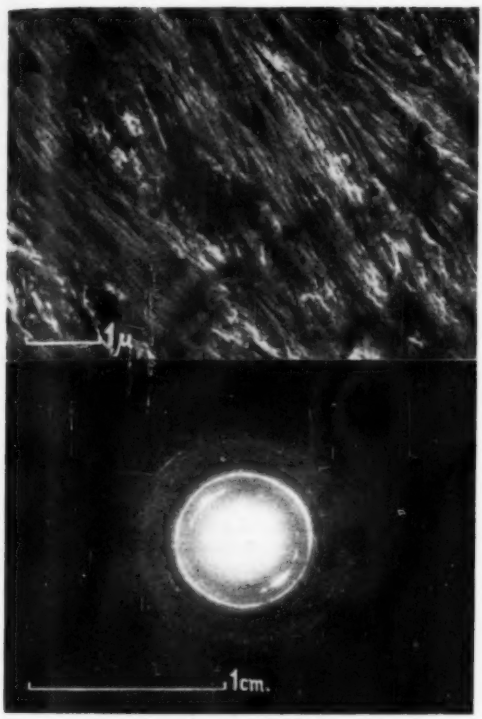

Fig. 1. Above, electron micrograph of fully mineralized unembedded human tooth enamel; below, selected area electron diffraction pattern from 1a. The pattern is characteristic of orientated apatite crystals. Kv. 100, pole piece 2, diffraction aperture 30 μ. Instrument constant $K = 1{\cdot}93$

direction of Dr. J. F. H. Custers and the late Dr. Paul Grodzinski.

These knives have now been under test for approximately five years without repolishing, and have successfully cut human tooth enamel without detectable damage to the edge. Fully mineralized human enamel is much harder than bone or dentine and has approximately the same degree of hardness as topaz. According to Gustafson and Kling[2] the Vickers micro-hardness number of enamel varies from 300 to 400 kgm./mm.^{2}. The Vickers micro-hardness value obtained from the specimen illustrated in this communication was 412 kgm./mm.^{2}. Because tooth enamel is extremely brittle, unsupported sections disintegrate when they come into contact with the collecting fluid. They may be collected, however, by first coating the surface to be cut with a thin film of 0·2 per cent 'Formvar' dissolved in chloroform. The surface is further supported by pressing a small piece of 'Sellotape' on to the surface to be cut. By holding the 'Sellotape' the section can be removed from the upper surface of the knife at the end of the cutting stroke. The specimen is then pressed face down on to a glass slide in a drop of 'Formvar'. When dry, the 'Sellotape' can be removed by dissolving the adhesive in petroleum ether.

Thick sections mounted in this way are suitable for phase-contrast microscopy. Alternatively, after removal from the knife the section may be floated 'Sellotape' side down in petroleum ether. When the adhesive dissolves, the 'Sellotape' backing sinks leaving the section supported by 'Formvar' floating; this may then be collected on an electron microscope grid. An example of an electron micrograph and an electron diffraction pattern obtained from a section of human enamel prepared in this way is shown in Fig. 1. It is possible that this technique might be usefully applied to other brittle substances.

The preparations were examined with a Siemens Elmiskop 1 electron microscope, generously provided by the Wellcome Trust. The work was further assisted by a grant from the Medical Research Council.

R. W. Fearnhead

Departments of Dental Histology and Anatomy,
London Hospital Medical College,
Turner Street,
London, E.1.

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RADIOBIOLOGY

Chelating Agents in the Treatment of Poisoning by Polymerizable Radioelements

The rate of reaction between a chelating agent and a hydrolysable polyvalent metal ion such as thorium (IV) is dependent on the degree of polymerization of the metal ion. It would be anticipated, therefore, that the degree to which a chelating agent might enhance the excretion rate of certain radioelements from the body would be variable even though factors such as dose and route of administration were kept constant. Accordingly, we investigated the effect of a strong chelating agent on the excretion of thorium-234 which was injected intravenously into rats in two different forms—a monomeric, diffusible form and a polymeric or colloidal form. The chelating agent used was diethylenetriaminepentaacetic acid, which forms an exceptionally strong chelate with thorium[1] (the 1 : 1 chelate has a $\log K = >27$).

A chemical demonstration of the difference in reactivity of the two forms of thorium toward the same chelating agent is easily given. For example, when a solution of thorium nitrate (pH = 2·5) is mixed with excess diethylenetriaminepentaacetic acid and the pH of the solution is increased to 7 with sodium hydroxide, the resulting solution remains clear because of the formation of the stable thorium–diethylenetriaminepentaacetic acid chelate. However, if an identical solution of thorium nitrate is neutralized before the diethylenetriaminepentaacetic acid is added, a precipitate forms which does not dissolve upon the addition of diethylenetriaminepentaacetic acid. Prolonged contact between the thorium precipitate—presumably an inorganic hydroxylated polymer[2]—and diethylenetriaminepentaacetic acid causes a slow dissolution of the precipitate over a period of many days.

In the metabolic experiments, two kinds of thorium solutions were used. The monomeric, or 'ionic', solution was prepared by adjusting a solution of carrier-free thorium-234 chloride with trisodium citrate and sodium hydroxide so that it contained 1 per cent citrate ion and had a final pH of 5·0. The polymeric solution was prepared with thorium-234 nitrate and stable thorium nitrate, adjusted with sodium hydroxide to pH 2·5 and a concentration of 1 mgm./ml. as carrier thorium. (The total concen-

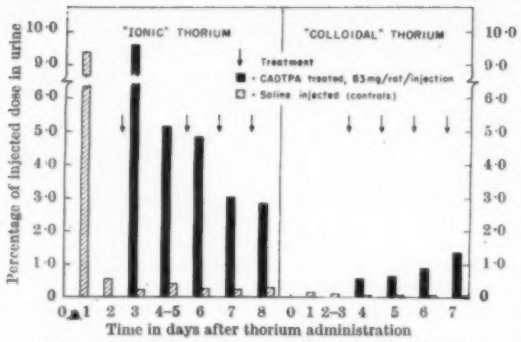
"IONIC" THORIUM
"COLLOIDAL" THORIUM
Treatment
■ - CADTPA treated, 83 mg/rat/injection
□ - Saline injected (controls)
Percentage of injected dose in urine
Time in days after thorium administration

Fig. 1. Effect of diethylenetriaminepentaacetic acid (CADTPA) on the urinary excretion of thorium in rats

tration of thorium in this solution therefore was 2×10^7 times as high as in the ionic solution.) This solution gave a well-defined Tyndall beam without evidence of visible precipitation. Mature Sprague–Dawley female rats, three rats per group, injected intravenously with 0·5 ml. of one of the two solutions at a level of 0·6 μc. per rat, were placed in individual metabolism cages. Two or more days later, the treated groups received a series of four daily intraperitoneal injections of 300 mgm./kgm. of the calcium chelate of diethylenetriaminepentaacetic acid, and the controls an equivalent volume of saline. A 24-hr. urine sample was collected daily throughout the experiment for each rat. The animals were killed by cervical fracture 24 hr. after the last treatment injection. The soft tissues and femurs were wet-ashed and counted for beta activity using an end-window Geiger–Müller counter.

The results show that diethylenetriaminepentaacetic acid caused a marked reduction of the body burden of thorium only when thorium was administered in the monomeric, or 'ionic', form[2]. Under these conditions, the thorium was deposited mainly in the skeleton. The total urinary excretion of thorium for the treated groups was 34 per cent of the injected dose as compared with 12 per cent for the saline controls. The pattern of this excretion is shown in Fig. 1. The first injection of diethylenetriaminepentaacetic acid increased the 24-hr. excretion from a control level of 0·5 per cent to more than 9 per cent of the injected dose. Subsequent injections effected significant increases, but the absolute amounts which were removed steadily diminished with each injection.

The effect of diethylenetriaminepentaacetic acid treatment on rats to which thorium had been given in polymeric, or 'colloidal', form is in marked contrast to this. The total urinary excretion was only 3·6 per cent of the injected dose in the treated group as compared with 0·2 per cent in the saline controls. The thorium was deposited mainly in the liver. In addition, the pattern of the excretion is quite different, as is shown in Fig. 1. Each subsequent injection of diethylenetriaminepentaacetic acid increased the urinary excretion of colloidal thorium to a level higher than that obtained with the previous injection.

It is unlikely that these differences in the distribution and excretion of the two solutions of thorium are a function of the difference in their concentration. It has been shown that thorium is closely related to plutonium in its metabolism[4] and that the distribution and excretion of plutonium are independent of dose[5]. Rather, the results shown in Fig. 1 can be explained as follows: when 'ionic' thorium is injected it is prevented from polymerizing because of rapid complex formation with body proteins. The administration of diethylenetriaminepentaacetic acid readily removes the bound but monomeric thorium by the formation of a diffusible chelate. Each subsequent injection of diethylenetriaminepentaacetic acid removes the labile, available thorium, but the amount of thorium available for removal diminishes with each treatment. However, in the case of 'colloidal' thorium, an insoluble polymer is deposited in the tissues. In the presence of sustained levels of diethylenetriaminepentaacetic acid within the tissues[6] the particles of thorium become slowly depolymerized. Consequently the amount of labile thorium, that is, thorium–diethylenetriaminepentaacetic acid chelate, increases with time so that subsequent injections of chelating agent result in an increase of excretion over the previous injection.

Details of these and other metabolic experiments involving the removal of thorium with chelating agents will be published elsewhere. The results cited here emphasize the point that the success of the use of chelating agents in the treatment of radioelement poisoning is dependent on the chemical and physical properties of the radioelement.

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Jack Schubert
Joan F. Fried

Division of Biological and
Medical Research,
Argonne National Laboratory,
Lemont, Illinois.

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Atmospheric Radiocarbon Activity in 1959

Attention has already been directed to the appreciable rise in the atmospheric radiocarbon activity following the testing of nuclear weapons[1–4]. On the basis of data obtained up till 1958, Broecker and Walton[1] estimated a rise in the atmospheric radiocarbon concentration in the northern hemisphere of 5 per cent per annum, though Münnich and Vogel[2] had arrived at a rate of rise of 3·2 per cent per annum. Measurements made in the summer of 1959, however, indicate a general level of activity greatly in excess of what might have been expected from either of these estimations.

In an attempt to evaluate the progressive rise in concentration in Britain over the past seven years, samples of annual oat crops were kindly supplied by the National Institute of Agricultural Botany, Cambridge, from its seed collection, and assayed for radiocarbon in this Laboratory. Oats have a very short growing season, and photosynthesize principally at the time of year when Münnich and Vogel[3] have demonstrated that the seasonal intensity of radiocarbon has reached its maximum. This variation has been ascribed to the rise in the tropopause in

Table 1

Sample No.	Year of harvest	Change from 1953 (per cent)
Q–436	1953	0·0 ± 0·5
Q–437	1954	− 0·7 ± 0·5
Q–438	1955	+ 1·2 ± 0·5
Q–439	1956	+ 3·0 + 0·5
Q–440	1957	+ 11·5 ± 0·6
Q–441	1958	+ 14·5 ± 0·6
Q–442	1959	+ 31·8 ± 0·7

summer, allowing part of the stratosphere to mix freely with the troposphere. The crops were grown in and around Cambridge, and thus the measurements from year to year are directly comparable.

If the level of activity of 1953, before any appreciable nuclear weapon testing had been carried out, is taken as the standard activity for subsequent years, the results (Table 1) may be expressed as a percentage variation from this value.

The large difference in activity between the summers of 1958 and 1959 must be attributed to the intense testing programmes undertaken late in 1958, and its delay in appearing in the atmosphere probably lies in the mechanism of the seasonal variation already referred to.

It is interesting to note that the activity of a sample of human neck hair cut in the late summer of 1959 was 16·5 per cent greater than the 1953 oat sample, and is thus considerably less than the activity of the 1959 oat crop. During the period of growth of this hair we can assume that the population was in the main still consuming the cereal and root crops of the previous season.

If the present suspension of nuclear weapon tests were to become permanent, the future level of the atmospheric radiocarbon activity, together with that of the surface ocean water, should furnish further valuable information about the exchange rates of carbon dioxide between the various phases of the radiocarbon reservoir[1,2].

E. H. Willis

University of Cambridge
Radiocarbon Dating Laboratory,
5 Salisbury Villas,
Station Road,
Cambridge.

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BIOLOGY

Implantation of Tissue Transplants in the Uteri of Pseudopregnant Mice

It was thought that transplants of tumour into the uterine cavities of pseudopregnant mice might simulate the invasive properties of the embryonic trophoblast sufficiently to throw light on the uterine reactions involved in the implantation of the embryo. Since the initial results of these studies, which are still in progress, appear to show clearly that the site of implantation is determined by the uterus, a preliminary report may be justified.

Mature females of strains *C*57 Black (pure line) and Lab. Grey (stock) mated with vasectomized males were used. The day on which the vaginal plug was found was taken as day 0 in the pseudopregnant cycle. The tumour used was the Harding–Passey[1] melanoma. Laparotomies were performed under ether anæsthesia and the tissue fragment inserted into the uterine lumen with a Bashford implantation needle (bore 0·625 mm.), ligatures being placed around the uterus above and below the transplant, which otherwise was expelled either through the incision or the cervix. The tumour was invariably transplanted into the right uterine horn and usually a fragment of the animal's own body-wall muscle into the other horn. Transplantations were performed from days 0 to 9 and the animals killed 2 days later. The uteri were then removed, fixed in Zenker's fluid and sectioned transversely. No differences were observed between the *C*57 Black and the Lab. Grey strains.

Table 1. Tumour Transplants in Pseudopregnant Lab. Grey and *C*57 Black Mice

Day of transplantation (after mating)	No. of experiments	No. showing implantation of tumour
0	4	0
1	6	0
2	6	(1*)
3	3	0
4	14	12
5	3	1
6	5	2 (+ 1*)
7	4	0
8	5	0
9	4	0
Dioestrus	3	0

* Animals in which only a few melanoma cells have invaded the mucosa.

It is apparent from Table 1 that implantation of the tumour in the uterine mucosa within the 2-day duration of the experiments was most frequent when the transplantation was effected on the fourth day of pseudopregnancy. Implantation was not observed after transplantation on days 0–2 or 7–9 of pseudopregnancy or during dioestrus. The autografts of muscle in the other uterine horn were made only

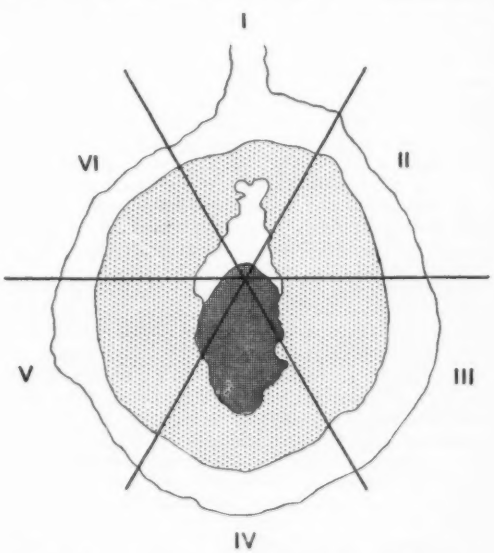

Fig. 1. Camera lucida outline diagram of tumour fragment (cross-hatched) implanting antimesometrially in the uterine mucosa (stippled) of a pseudopregnant mouse. The sectors employed in Tables 2 and 3 for defining the position of attachment are superimposed

Table 2. Uniformity of antimesometrial Implantation of the Tumour in Lab. Grey and C57 Black Mice

Experiment No.		Day of transplantation	Sectors of uterus containing implant					
			I	II	III	IV	V	VI
Lab. Grey	1	4			+	++	+	
	2	4			+	++	+	
	3	4				++	+	
	4	4			+	++	+	
	5	4			+	++	+	
	6	4			+	++		
	7	4			++	+		
	8	4			+	++	+	
	9	4			+	++		
	10	5				++		
	11	6				+	+	
C57	12	4			++	+		
	13	4			+	+		
	14	4			+	++		
	15	6				+	++	

++, Implantation extending across whole sector; +, implantation spreading into sector.

Table 3. Uniformity of Antimesometrial Implantation of Muscle Tissue in Lab. Grey and C57 Black Mice

Experiment No.		Day of transplantation	Sector of uterus containing implant					
			I	II	III	IV	V	VI
	—*	3			++	+		
	—*	3			+	++	+	
Lab. Grey	1	4			+	++	+	
	2	4			+	++	+	
	3	4			+	++	+	
	4	4				++	+	
	—*	4			+	++	++	
	—*	5				+	++	
	—*	5					++	+
C57	12	4			+	++		
	13	4				++	+	
	14	4			+	++	+	
	—*	5				++	++	+
	15	6			+	++		

—*, Experiments where the tumour tissue did not become implanted, but the control muscle tissue did.

on days 3–6 of pseudopregnancy and failed to become implanted in only one instance in fifteen experiments.

The melanoma or muscle tissue when it became implanted did so invariably on the antimesometrial side of the lumen as does the normal embryo in the mouse. As a rule the tumour had penetrated the epithelium and deeply invaded the mucosa, the camera lucida outline diagram of a section in Fig. 1 being typical. The muscle tissue became attached to the mucosa, usually with destruction of the epithelium but without further invasion of the mucosa. The position and extent of the attachments are represented in Tables 2 and 3 by dividing the camera lucida outline drawings of the median transverse section into 6 sectors, as in Fig. 1, sector I being mesometrial and sector IV antimesometrial.

The tissue fragments implanted were many times larger than a normal embryo, being at least five times the diameter, yet whether homografts of melanoma or autografts of muscle were employed, implantation occurred with remarkable uniformity antimesometrially in precisely the region where an embryo would have become implanted. It is difficult to escape the conclusion that the antimesometrial site of implantation is determined by the uterus and that the properties of the mucosa at the appropriate stage of pseudopregnancy are such as to encourage the attachment of all these diverse tissues. The work of McLaren and Michie[2] has shown that the siting of implantations along the length of the uterus is not predetermined.

Other aspects of this work still in progress, such as the induction of the decidual reaction, together with a full report of the histological findings, will be published later.

I am indebted to Prof. F. W. R. Brambell for his advice and encouragement, to Prof. A. Haddow, of the Chester Beatty Institute, for supplying the tumour, and to the staff of this Department for technical assistance. The work was performed during the tenure of a Research Studentship of the Department of Scientific and Industrial Research.

Ian B. Wilson

Department of Zoology,
University College of North Wales,
Bangor, Caernarvonshire.
Nov. 20.

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Effect of Soil Drying on the Viability of *Heterodera major* Cysts

Winslow[1] has shown that dried cysts of *Heterodera major* do not hatch well. He found that more than two thousand larvæ hatched from cysts recovered from wet soil, whereas only seven larvæ hatched from cysts which had been recovered from dried soil. Hesling[2], in an experiment carried out at Rothamsted, confirmed Winslow's results, and concluded that this eelworm is quite sensitive to drying.

Cysts of *H. major* were found on the roots of volunteer oat plants which were growing in discarded infected soil. Prior to being discarded, this infected soil had been air-dried in the laboratory and, since cysts were found on the roots of oat plants growing in this soil later, it would appear that the drying of the soil did not seriously affect the viability of the cysts in it.

Early in January 1955, a quantity of infected soil was placed in a heap and was thoroughly mixed to ensure uniformity in cyst-distribution throughout. A microplot (in the field) was filled with this mixture and at certain intervals over a period of fifteen months a portion of soil, sufficiently big to fill ten test-tubes, was taken from the microplot and was air-dried. When the soil was dry, the ten test-tubes were filled with it, and it was thus stored in this air-dried condition in the laboratory until sowing time. This gave lots of infected soil which had been air-dried for periods of 1, 3, 6, 9, 12 and 15 months. Ten tubes containing infected soil which was not air-dried and ten tubes containing non-infected soil were included in the experiment, also, and were used as controls.

On April 16, 1956, all tubes were sown with oats (variety Sun II), and were plunged in a bed of peat-moss in the field.

By the middle of June, cysts of *Heterodera major* were found to be quite plentiful on the roots of plants growing in the infected soil which had been air-dried for periods of one and three months and in the infected soil which had not been air-dried. It must be emphasized that the soil, when dried for these periods, was 'dust-dry', and was certainly dry enough for cyst extraction using the Fenwick apparatus.

Despite repeated examinations, cysts were not observed on the roots of plants growing in infected soil which had been air-dried for periods of 6, 9, 12 and 15 months. It was concluded that, if infected soil is air-dried, and kept in an air-dried condition for a period of 6 months or longer, the cysts lose their viability and their contents become incapable of hatching and invading the roots of a host-plant.

It is interesting to record that the effect of drying on the viability of the cysts was reflected in the growth of the plants. The plants growing in soil which had been air-dried for periods of 6, 9, 12 and 15 months were comparable in size and vigour with plants which were growing in tubes of non-infected soil, and showed no symptoms of eelworm attack. On the other hand, plants growing in the infected soil which had been air-dried for periods of 1 and 3 months showed symptoms of severe eelworm attack and were very dwarfed and diseased-looking.

The hatching experiments of Winslow[1] and of Hesling[2] suggest that cysts of *H. major* are far more sensitive to drying than the above-mentioned observations made by me found them to be. It is possible that the effect of drying becomes more pronounced when the cysts are removed from the soil, a factor which might explain this apparent contradiction in results.

J. J. Duggan*

Agricultural Zoology Department,
University College,
Dublin.

*Now Senior Research Officer, Nematology Department, Agricultural Institute, Dublin.

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Albinism in *Cryptomys hottentotus* Lesson, 1826

In an earlier communication[1] I reported the occurrence of what appeared to be an albino specimen of the above species of mole-rat. I am grateful to Miss D. Watson for a further and similar example, collected on November 12, 1958, at a point within a few hundred yards of where the first animal was taken.

As in the previous instance the mammal was taken on the surface, and was examined while still alive. In all respects the coloration was similar to that previously described, except that the dietetic staining was not so pronounced. The specimen, an adult male, was preserved entire, and revealed the following body measurements: head and body, 130·0 mm.; tail, 15·0 mm.; hind foot s.u., 22·0 mm. The cheek teeth were again simple, 4/4.

Previously I stated that normal specimens of *C. hottentotus* at Inyanga possessed a "variable white head-spot which is sometimes vestigial". Since this was written I have handled specimens of adults, taken side-by-side with head-spotted examples, in which the head-spot has been entirely absent. Thus it seems that Inyanga specimens are the antithesis of those described by Roberts[2], who notes the position regarding head-spots as "white frontal spots are not infrequent, but abnormal". The converse may be said to be true at Inyanga.

It might be of further interest to note that while the tail normally has a length of about 11·0–22·0 per cent of head and body-length, some specimens may have tails as long as 30·0 per cent of head- and body-measurement.

Peter St. J. Turnbull-Kemp

Rhodes Inyanga Estate and National Park,
Rusape,
Southern Rhodesia.

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Quantitative Immuno-Analysis in Gel Plates

I cannot let pass unchallenged certain assertions made by Wright[1] in his communication in *Nature* of May 2, 1959, with reference to my method for quantitative immuno-analysis in agar gel plates[2]. Dr. Wright states: "Although there are several methods available, none of them is easily applicable to a multi-antigenic system, and in addition the concentrations of the proteins can only be estimated separately, even under favourable conditions, at ±10 per cent and more usually ±50 per cent".

The entire joy of the quantitative plate method, as outlined in my original communication[2] and since extended[3-5], is that it permits the ready titration of multi-antigenic systems and the simultaneous assay of individual antigens, often on a single plate. It has the further advantage of not requiring titration to an end-point, assays being readily performed by comparison of precipitation patterns.

As for the sensitivity of the technique, claims below ±10 per cent have not been made simply because we have never felt the need for greater accuracy and have been content to use, at the narrowest, 1·1× serial dilutions. A narrower dilution gradient, as used by Wright in his technique, could well produce accuracies better than ±10 per cent.

J. G. Feinberg

Bencard Allergy Research Unit,
Beecham Research Laboratories,
Brockham Park,
Betchworth, Surrey.

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MICROBIOLOGY

Exogenous Respiration in *Nitrobacter*

During the course of an investigation of the oxidation of nitrite by the nitrite-oxidizing bacterium, *Nitrobacter*, it was noted that the addition of sodium nitrite but not sodium nitrate to heavy cell suspensions caused the immediate appearance of reduced cytochrome bands at 590, 551 and 513 mμ when viewed in the hand spectroscope[1]. This phenomenon, which has been noted previously by Lees and Simpson[2], is due to the passage of electrons through the respiratory chain when nitrite is oxidized to nitrate. Whether or not nitrifying bacteria respire in response to substrates other than their energy source has been disputed, although Bömeke[3] reported a slight stimulation of the respiration of *Nitrobacter* when certain metabolites were added to cell suspensions in the Warburg respirometer. If some reduced substance is oxidized by resting cells and if a cytochrome-linked pathway is employed for the oxidation of all substances which can permeate the cell, then any substance which might increase the rate of respiration in the Warburg respirometer should also cause a reduction of the cellular cytochromes. The validity of this rationale was tested in the following manner.

Cultures grown for 4–8 days at 25° C. on a reciprocating shaker in a mineral medium (gm./l.: sodium nitrite, 1·0; sodium chloride, 0·5; dihydrogen potas-

sium phosphate, 0·44; dipotassium hydrogen phosphate, 2·88; crystalline magnesium sulphate heptahydrate, 0·3; ferrous sulphate, 0·003; sodium carbonate, 1·0; *p*H to 7·0–7·2) were harvested by centrifugation and washed until free of nitrite. Heavy cell 'creams' were thoroughly aerated for 30–60 min. and then observed in the Hartridge reversion spectroscope (slit-width 125μ). The spectrum obtained was that of an oxidized cytochrome system, and it remained oxidized for at least 30 min. after the cessation of aeration. Various substrates (0·1 ml. of neutralized 0·05 *M* solutions) were pipetted into the cell suspension and it was observed for 30 min. for spectral changes. As shown in Table 1, only sodium nitrite and formate caused a reduction of the cellular cytochromes. The bands appeared immediately (within 10 sec.) and were in identical positions for both substances.

Table 1. The Effect of Selected Metabolites on the Respiration of *Nitrobacter*

Addition	Reduced cytochrome bands appearing, mμ	Q_{O_2} (28° C.)
None	None	2·4
Sodium nitrite ..	590, 551, 513	51·7*
Sodium nitrate ..	None	N.T.
Sodium formate ..	590, 551, 513	10·8*
Sodium acetate ..	None	4·8†
Sodium lactate ..	None	2·2
Glucose	None	1·8
Sodium citrate ..	None	1·3

N.T. = Not tested.

* No lag.
† After 50-min. lag during which respiration was equivalent to endogenous.

The effect of these substances on the oxygen uptake of washed cells was tested by conventional techniques in the Warburg respirometer (final concentrations: substrate, 25 μ*M*; *p*H 7·2 phosphate buffer, 400 μ*M*; potassium hydroxide in the centre well). Oxygen uptake was followed for a total of 2 hr. after tipping in the substrate, although nitrite was completely oxidized within the first 45 min. It may be noted in Table 1 that of the substances tested, only formate and nitrite readily stimulated oxygen uptake. Thus formate is a metabolizable substrate for intact *Nitrobacter* cells. Whether the slight increase in oxygen uptake with acetate is significant is not known—however, it lies within the range of experimental error. Thus only those substances which evoked the rapid reduction of cellular cytochromes caused an increase in uptake of oxygen. The spectroscopic method, therefore, appears to offer a rapid and convenient way to survey potential substrates for *Nitrobacter*.

These preliminary results suggest that the following interesting speculations be tested: The autotroph, *Nitrobacter*, differs from better-known heterotrophic bacteria in that it is not permeable to many of the usual metabolic intermediates. This is reflected by the lack of effect of common metabolites on respiration. It is reasonable to postulate that many of the common enzyme systems are operative within the cell, but the use of intact cells gives no evidence of this potentiality due to the impermeability to the required substrate. Cell-free preparations, however, should possess many of the enzyme systems which catalyse the utilization of some of the substances listed in Table 1. These hypotheses are now being tested experimentally by spectroscopic and manometric techniques.

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W. S. Silver

Department of Bacteriology,
College of Agriculture,
University of Florida,
Gainesville, Florida.

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Shape of Ultra-violet Inactivation Curves of Transforming Deoxyribonucleic Acid

In analysing the effects of radiation on biological systems, it is customary to plot the logarithm of the surviving fraction (or of the fraction of the biological activity remaining) against the applied dose. When such a plot gives a straight line passing through the origin, the radiation sensitivity of the system may be characterized by a single constant, which is conveniently taken as the negative slope of the logarithmic inactivation curve. Such a curve is consistent with the assumption that the inactivation results from independent, single events (inactivating 'hits') occurring at random[1].

When the inactivation of bacterial transforming deoxyribonucleic acid by ionizing or ultra-violet radiation is analysed by this procedure, the curves are not straight lines, but bend steadily in the direction of shallower slopes as the radiation dose increases[2–4]. 'Multicomponent' curves of this character require two or more constants for their characterization. They are obtained if the irradiated population is heterogeneous in its sensitivity to radiation[1], each class being inactivated according to a straight line curve (exponential law). In such cases the plot becomes a straight line at high doses where the survivors consist chiefly of members of the most resistant class. These curves can also result from suitable heterogeneity in the substructure of each individual. Deoxyribonucleic acid inactivation results for ionizing radiations have usually been interpreted in terms of one of these schemes, with attempts to determine the 'limiting slope' at high radiation doses corresponding to one resistant class of deoxyribonucleic acid molecules or one class of molecular substructures[5,6].

In semi-logarithmic inactivation curves for *Hemophilus influenzae* transforming deoxyribonucleic acid exposed to 254 mμ ultra-violet radiation, we find a steadily decreasing curvature at high doses[4], in agreement with other workers[3]. These inactivation curves, for all the genetic markers tested, can be converted into straight lines over a wide range of high doses by plotting the logarithm of the transforming activity against the *logarithm* of the ultra-violet dose (instead of against the ultra-violet dose itself) (unpublished work of S. H. G.). Such a relation on a log–log plot is incompatible with a straight line on a semi-log plot, and indicates a power-law inactivation at these doses rather than some variety of exponential law.

Preparation, ultra-violet inactivation and assay of transforming deoxyribonucleic acid were carried out as detailed in ref. 7 (except that the concentration of deoxyribonucleic acid in contact with bacterial

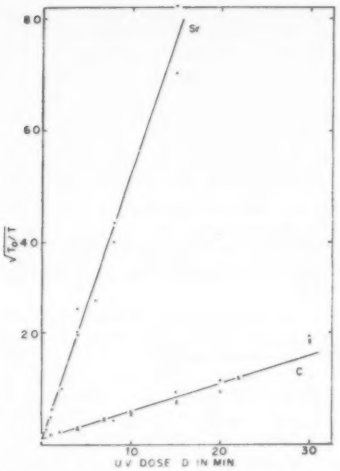

Fig. 1. Reciprocal square root of the fractional transforming activity $\sqrt{(T_0/T)}$ vs. the radiation dose D (in min. at 1,650 ergs mm.$^{-2}$ min.$^{-1}$ of 254 mμ radiation). T_0 is the transforming activity before irradiation, and T the activity afterwards of the genetic markers, streptomycin resistance (Sr 2,000 γ/ml.) and cathomycin resistance (C 2·5 γ/ml.). Deoxyribonucleic acid concentrations in contact with cells during transformation were as follows: (●) 5×10^{-4} γ/ml.; (○) 5×10^{-3} γ/ml.; (×) 10^{-2} γ/ml.

cells during transformation was as indicated in the caption of Fig. 1). The ultra-violet dose-rate in ergs/mm.^{2}/min. was estimated from the rate of inactivation of $T2$ bacteriophage[8]. For all markers tested, a plot of the reciprocal square root of the transforming activity against dose leads to straight lines over the entire dose-range (Fig. 1), implying an inverse square law:

$$T/T_0 = 1/(1+CD)^2 \qquad (1)$$

Here T_0 is the initial transforming activity of a preparation, T is the activity after exposure to a radiation dose D, and C is a constant depending on the particular genetic marker under study. This single constant, which may be used to characterize the sensitivity to radiation of the marker, is equal to minus one-half the initial slope of a semi-logarithmic plot of the same results. The entire curve may be used, however, in determining it, instead of only the initial portion. From the slopes of the lines in Fig. 1, the relative sensitivities to ultra-violet radiation of the streptomycin-resistance and cathomycin-resistance markers of *H. influenzae* are easily specified as being in the ratio of 10 to 1, a fact which would be difficult to state quantitatively from the usual presentation. Genetic markers for erythromycin resistance and viomycin resistance (which also give straight lines on an inverse square root plot) have sensitivities very close to that of the cathomycin marker.

The straight line region, of slope minus 2, which appears on a log–log plot at high doses, corresponds to the entire dose-range in which $CD \gg 1$; that is, to the region in which the transforming activity is less than about 10 per cent of that preceding irradiation.

The absoluteness with which relation (1) holds can be determined only by extensive experience. Experiments covering the low dose-range more thoroughly than those reported here show that it describes inactivation very well in this region. At the very highest doses it may perhaps predict a slower inactivation than is actually found by experiment, since the points shown for cathomycin resistance at 25 and 30 min. (41,000 and 50,000 ergs/mm.^{2}) lie slightly off the theoretical fitted curve. This discrepancy is not clearly present in all experiments and its reality is not certain.

At present we offer no theoretical interpretation of relation (1), which is entirely empirical. Its simplicity suggests that the complex ultra-violet inactivation curves observed on semi-logarithmic plots may, after all, arise from a relatively simple mechanism, although one which possibly differs from the type of inactivation envisioned by elementary target theory. The simpler and apparently more appropriate manner of presenting ultra-violet inactivation data which now becomes available should facilitate studies aimed at determining this mechanism.

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Claud S. Rupert
Sol H. Goodgal

Department of Biochemistry,
Johns Hopkins University,
School of Hygiene and Public Health,
Baltimore 5, Maryland.
Sept. 2.

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STATISTICS

Performance of a Stochastic Net

Stochastic nets have been proposed by several authors[1–3] as models of cognitive activity. So far, these have been dominated by heuristic constraints designed to show some analogy with the nervous system or merely for ease of computation. An adequate mathematical theory has, as was recognized by Uttley[4], to show how such a device can learn relations.

In the following I give a model which with a simple and natural constraint has the ability to detect linear relations and record their components in a principal axis frame of reference; as such it has obvious relations with well-known topics of linear analyses, namely, multiple factor[5], and prediction and filtering[6] theory.

Consider a collection of n cells i; let α_i, α^i, be their inputs and outputs respectively and W_{ij} the strength of connexion from cell j to i such that if α_i^S is the input from sensory field S to cell i, we have: $\alpha_i = \alpha_i^S + W_{ij}\,\alpha^j$ (summation over repeated indices) assuming unit linear amplification for each cell i (that is, $\alpha^j = \delta^{ji}\alpha_i$); the effective amplification with positive feedback W_{ii} is $(1-W_{ii})^{-1}$ so that the above can be written:

$$\alpha_i^S = (\delta_{ij} - W_{ij})\,\alpha^j = A_{ij}\alpha^j \text{ say} \qquad (1)$$

W_{ij}, α_i^S may be positive or negative according as their synaptic endings are excitatory or inhibitory.

Consider n outputs of receptors from a sensory field S such that the covariance:

$$C_{ij} = \lim_{T\to\infty} C_{ij}(T) = \lim_{T\to\infty} \frac{1}{T}\int_0^T \alpha_i^S(t)\,\alpha_j^S(t)\,dt \qquad (2)$$

exists. The structure of this matrix reflects the simplest properties of the field S, namely, if the rank $r<n$ then there will be $n-r$ linear relations between the α_i^S. If now we can arrange that:

$$\lim_{t\to\infty} \sum_k A_{ik}(t)\,A_{jk}(t) = C_{ij} \qquad (3)$$

then we shall have a description of the field $S(t)$ in terms of n outputs $\alpha^j(t)$ which are orthonormal in the sense that:

$$\lim_{T\to\infty} \frac{1}{T}\int_0^T \alpha_i(t)\,\alpha_j(t)\,dt = \delta_{ij} \qquad (4)$$

and of which therefore $n-r$ must be arbitrary, that is, generated by the net itself. Were it not for this, which will allow us to distinguish them from the r outputs representing $S(t)$, we should have gained nothing from the transformation (1).

To realize (3), let us put:

$$t\frac{\partial A}{\partial t}ik + \frac{1}{2}A_{ik} = \frac{1}{2}\alpha_i^S\alpha_k \qquad (5)$$

Multiplying through by A_{jk}, using the symmetry of C_{ij} and summing over k it is easy to show that this satisfies (2) and (3). However, (3) only determines A_{ij} up to an orthogonal transformation corresponding to an arbitrary rotation of the orthogonal frame; the additional condition:

$$_k\Sigma\, A_{ki}A_{kj} = \lambda_i\,\delta_{ij} \qquad (6)$$

determines the principal axis solution, where λ_i are the proper values of C_{ij}. If (6) holds, then we have:

$$A_{ij}\,\alpha_i^S = \lambda_j\,\alpha_j \qquad (7)$$

multiplying (5) by A_{ij} and summing this time over i, one can check that (6) follows.

Using (1) and (4), the corresponding condition for the connexions is:

$$t\frac{\partial W}{\partial t}ij + \frac{1}{2}W_{ij} = \frac{1}{2}(\alpha_i - \alpha_i^S)\alpha_j \qquad (8)$$

which means that the (negative) connexion between two cells is proportional to the correlation between the activity of the cell from which the connexion is coming and the stimulus on the cell to which it is going. For the feedback of a cell to itself the same is true of the reciprocal gain. This shows that if α_j is uncorrelated with any of the α_i^S, then the cell j is effectively isolated and its gain rises to an infinite value such that α_j is a self-generated signal of unit variance. Under these conditions an indefinitely flat peak in the frequency response will produce a single-frequency sinusoidal output. If no two of these frequencies are exactly equal, then (4) will certainly be satisfied. It is not unreasonable to compare this behaviour with the α-rhythm of the inattentive brain. Furthermore, the principal axes solution in which the co-ordinate axes are determined by successive extrema of the stimulus vectors is the one most indicated by physiological evidence.

An example of the application of such a net would be the following: suppose we have n audio receptors responding to a frequency band $\Delta\nu_i$, and let their (rectified and smoothed) outputs be $\alpha_i^S(t)$. For a sensory field such as normal speech, the latter will be highly correlated, so that a smaller number r will be able to convey the information. In fact, just such a procedure has been used[7] in a speech communication channel known as the 'Vocoder'. For example, with $\Delta\nu_i = 15$ c./s., $n = 16$, we can have $r = 10$ without loss of fidelity.

However, the significance of the net as a model of local brain activity would appear to be the following. If we drop the rigour implied in the infinite limit of (2), then what the net does for us is to factorize a sensory field into a part which has no constancy in time and a part which is invariant over a period of time T, and which is expressed in the values of the A_{ij}. Over longer periods of time, the latter may vary and could be the subject of analysis by a net of longer time constant. In this way knowledge is possible in spite of the conditional and incomplete nature of all relations.

Note added in proof. To obtain the principal axis solution uniquely some modification of (5) is required; it will be given elsewhere.

M. C. Goodall

Cornell Computing Center,
Ithaca, New York.

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Standardized t

Students' t may be standardized by expressing it in units of its own standard deviation; thus, $t_s = t\sqrt{\{(\nu-2)/\nu\}}$, where ν is the number of degrees of freedom. The procedure reduces the variability of the tails with ν for a given probability, P, and in particular, for $P = 0{\cdot}05$ and $\infty \geqslant \nu \geqslant 4$, t_s lies between $1{\cdot}96$ and $2{\cdot}0$ whereas t lies between $1{\cdot}96$ and $2{\cdot}776$. This finding extends the applicability of certain 'large' sample methods down to 4 degrees of freedom. The standardizing is extremely simple to apply—merely divide the error sum of squares by $\nu - 2$ instead of by ν and use this value to calculate the appropriate standard error (SE). For example, if d is the difference between two means and $d \geqslant 2\,SE$, $P \leqslant 0{\cdot}05$, that is, the difference is significant at the 5 per cent level. If $d < 1{\cdot}96\,SE$, $P > 0{\cdot}05$ and the difference is not significant. Between these two limits of d, $P \simeq 0{\cdot}05$; actually $0{\cdot}0535 > P > 0{\cdot}0455$. Again, $\pm 2\,SE$ will give a close approximation to 95 per cent confidence limits (actual value between 95 and 95·45 per cent). When the formula is applied to the correlation coefficient, r, we find that r is significant at the 5 per cent point if $r \geqslant 2/\sqrt{(\nu+2)}$.

John B. de V. Weir

The University,
Glasgow, W.2.

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 22

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Are We Making the Best Use of Research Resources" opened by Mr. L. Rotherham.

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Place of Management Studies in the Training of an Engineer".

Monday, February 22—Friday, February 26

ENGINEERING MATERIALS AND DESIGN EXHIBITION AND CONFERENCE (at Earls Court, London, S.W.5).*

Tuesday, February 23

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 1.30 p.m.—Dr. Donald Hunter: "Historical Perspective in Science and Medicine".

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. A. Bell: "Corrosion and Fires in Marine Boiler Air Preheaters".

INSTITUTE OF PHYSICS, ELECTRONICS GROUP (at 47 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. W. M. Lomer: "Magnetic Properties of Alloys".

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. H. E. Rose: "Vibratory Ball-mills".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. R. S. Doll: "Epidemiological Methods in Leukæmia Research".* (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on February 25 and March 1.)

UNIVERSITY OF LONDON (in the Chemistry Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. A. B. Gray: "The Place of Physiology in University Education".*

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Courses for Electrical Technicians" opened by Prof. M. W. Humphrey Davies.

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Water Lubrication".

BRITISH INTERPLANETARY SOCIETY (joint meeting with the LONDON BRANCH of the INSTITUTION OF PLANT ENGINEERS, at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. R. H. B. Forster and Mr. L. Breen: "Some Problems Encountered in the Design of Large Rocket Test Beds".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. A. J. Troughton: "Relationship between Theory and Practice in Structural Problems".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. D. F. White and Mr. L. E. Taylor: "Backscatter Method of Wall Thickness Measurement"; Mr. M. V. James: "Ultrasonic Resonance Method of Wall Thickness Measurement".

Wednesday, February 24

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. R. R. Race: "The Genetics of Blood Groups".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Prof. A. C. B. Lovell, F.R.S.: "The Exploration of Outer Space" (Trueman Wood Lecture).

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. E. Strauss: "The Structure of the English Milk Industry".

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. E. H. Hubbard: "Comparison of Different Methods of Fluid-atomizing Oil Flames", and "The Effect on Flame Emissivity and Radiation of the Addition of Carbon Black to Liquid Fuels" (Performance Trial No. 8 of the Joint Committee of the International Flame Research Foundation).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. A. L. Cullen: "Applications of Microwaves".

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. H. Wilkinson: "Language and the Elementary Particles".* (First of three Special University Lectures in Physics. Further lectures on February 25 and 26.)

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the MICROBIOLOGY GROUP, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "New Methods of Factory Hygiene in the Food Industry". Mr. G. A. Thomas: "Chemical Sterilants in the Food Industry"; Mr. W. J. Blois-Johnson: "Hygienic Considerations in Food Plant Design".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Dr. T. D. Whittet: "Pharmaceutical Fellows of the Royal Society".

Thursday, February 25

CENTRAL LONDON PRODUCTIVITY ASSOCIATION, WORKING RELATIONS SUB-COMMITTEE (at 21 Tothill Street, London, S.W.1), at 2.30 p.m.—Meeting on "The Factories' Act and the Work of the Factory Inspectorate".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. F. W. R. Brambell, F.R.S., Mr. W. A. Hemmings, Prof. C. L. Oakley, F.R.S., and Mr. R. R. Porter: "The Relative Transmission of the Fractions of Papain Hydrolysed Homologous γ-Globulin from the Uterine Cavity to the Fœtal Circulation in the Rabbit"; Prof. D. Lewis, F.R.S.: "Genetic Control of Specificity and Activity of the *S* Antigen in Plants".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. G. Kuschel: "The Natural History of Juan Fernandez Islands"; Dr. M. W. Holdgate: "The Natural History in the South Chilean Islands".

Friday, February 26

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Alexander Kennedy: "The Scientific Lessons of Brainwashing".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

KNOOP RESEARCH FELLOW (graduate in medicine or science), to work on cerebral enzymatic aspects and on the presence of chemical abnormalities in hereditary degenerative cerebral conditions—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1 (February 25).

LECTURER (graduate of a British university, preferably with teaching experience) IN PURE AND APPLIED MATHEMATICS in the Department of Building, Structural Engineering and Surveying—The Secretary, Hammersmith College of Art and Building, Lime Grove, London, W.12 (February 25).

HORTICULTURALIST (male), to take charge of the Insecticide Department glasshouses—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (February 29).

KEEPER (graduate or holder of the Diploma of the Museums Association, a good knowledge of Western European archæology, and preferably an interest in the archæology of the Eastern Mediterranean) OF ARCHÆOLOGY—The Director, Leeds City Museum, Park Row, Leeds 1 (February 29).

LECTURER IN THE DEPARTMENT OF PSYCHOLOGICAL MEDICINE—The Secretary, The University, Edinburgh (February 29).

RESEARCH STUDENT IN METALLURGY, for postgraduate work on liquid metals—The Registrar, King's College (University of London), Strand, London, W.C.2 (February 29).

LECTURER IN SOCIAL SCIENCE, a LECTURER IN SOCIAL PSYCHOLOGY or IN SOCIOLOGY, and 2 LEVERHULME RESEARCH FELLOWS—The Registrar, University College, Singleton Park, Swansea (March 5).

LECTURER (with a good honours degree and teaching experience) IN CHEMISTRY at the Nigerian College of Arts, Science and Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (March 5).

RESEARCH FELLOW IN MATHEMATICS—The Secretary of University Court, The University, Glasgow (March 5).

SENIOR LECTURER/LECTURER IN FUEL TECHNOLOGY in the School of Mining, Engineering and Applied Geology, University of New South Wales—Agent General for New South Wales, 56–57 Strand, London, W.C.2, and The Bursar, Post Office Box 1, University of New South Wales, Kensington, N.S.W., Australia, marking envelope "University Appointment" (March 5).

ASSISTANT PROFESSOR (with high academic qualifications in physics or mathematics, and with experience in (*a*) experimental reactor physics, (*b*) the design of critical and subcritical experiments, and (*c*) safety precautions associated with (*a*) and (*b*), and with the use of highly radioactive sources) OF NUCLEAR SCIENCE AND TECHNOLOGY at the Royal Naval College, Greenwich—The Civil Service Commission, 17 North Audley Street, London, W.1, quoting S./5101/60 (March 10).

LECTURER (with a degree in veterinary science, together with postgraduate experience) IN THE DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 12).

PHYSIOLOGIST or BIOCHEMIST (honours graduate in physiology or biochemistry, with relevant postgraduate research experience) IN THE CATTLE RESEARCH LABORATORY, Division of Animal Genetics, Commonwealth Scientific and Industrial Research Organization, Rockhampton, Queensland, Australia, for the study of the adaptation of cattle to a tropical and subtropical climate—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 231/5 (March 12).

LECTURER IN PHYSICS (COSMIC RAY GROUP)—Prof. P. M. S. Blackett, F.R.S., Physics Department, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7 (March 14).

BIOCHEMISTS (2) (honours graduates in science (or equivalent qualifications), with suitable postgraduate research experience, preferably to Ph.D. level) IN THE DIVISION OF BIOCHEMISTRY AND GENERAL NUTRITION, Commonwealth Scientific and Industrial Research Organization, Australia, for research into the intermediary metabolic processes of ruminant animals—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 250/92 (March 15).

LECTURER IN ZOOLOGY—The Secretary, The Queen's University, Belfast (March 15).

LECTURERS IN PURE MATHEMATICS—The Secretary, The Queen's University, Belfast (March 21).

SENIOR LECTURER (with special qualifications in hydraulics) IN CIVIL ENGINEERING at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 21).

CHAIR OF PSYCHOLOGY—The Registrar, The University, Leicester (March 26).

LECTURER IN PSYCHOLOGY—The Registrar, The University, Leicester (March 26).

CENTRAL ELECTRICITY GENERATING BOARD RESEARCH FELLOW (of British nationality, and by October 1960, taken the degree of Doctor of Philosophy, or have had equivalent research experience), for research in the physical sciences, including metallurgy, engineering or mathematics—The Master Elect, St. Catherine's College Planning Office, 42 Banbury Road, Oxford (March 31).

PROFESSOR OF EDUCATION at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (March 31).

SECRETARY (with senior administrative experience in or with Governments, preferably in departments connected with science, agriculture or forestry; also overseas experience or experience in inter-commonwealth relations, and preferably aged 50 to 55 with a scientific background or training, and fluency in some foreign languages) TO THE EXECUTIVE COUNCIL OF THE COMMONWEALTH AGRICULTURAL BUREAUX—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (April 15).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

TURNER AND NEWALL RESEARCH FELLOW IN ENGINEERING, INORGANIC CHEMISTRY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

LECTURER (preferably with experience of terrestrial ecology) IN ANIMAL ECOLOGY—The Secretary, University College London, Gower Street, London, W.C.1 (May 1).

NATIONAL RESEARCH COUNCIL OF CANADA POSTDOCTORATE FELLOW IN APPLIED MATHEMATICS—G. R. Magee, Department of Pure and Applied Mathematics, University of Western Ontario, London, Ontario, Canada (May 1).

RESEARCH SCHOLARS (with a good honours degree or graduate member of the Royal Institute of Chemistry) IN THE DEPARTMENT OF CHEMISTRY, for research in higher dispersion infra-red spectroscopy for Ph.D. or M.Sc. degree—The Professor of Chemistry, The University, Reading (May 2).

HOLLERITH RESEARCH STUDENT (graduate of any university), to undertake research in automatic computing or in a subject allied thereto—The Secretary to the Academic Council, The Queen's University, Belfast (June 1).

AGRICULTURAL CHEMIST (male, with a good honours degree in agricultural chemistry, with some postgraduate experience, preferably in the tropics) in Sierra Leone, to advise the Director of Agriculture on specific problems and for investigational studies in the field, including the laying out of field trials and their statistical analysis and interpretation—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/15/04/T.

ASSISTANT or ASSOCIATE PROFESSOR OF MATHEMATICS—The Principal, Victoria College, Victoria, B.C., Canada.

BIOCHEMIST (Senior or Basic grade) to the Rochdale Group of Hospitals, to work under the Consultant Pathologist and to carry out biochemical work for the hospitals and clinics of the Group—The Group Secretary, Rochdale and District Hospital Management Committee, Central Offices, Birch Hill Hospital, Rochdale, Lancs.

CHEMIST (Ph.D.), to supervise rock and soil analytical laboratory, a CHEMIST (honours B.Sc.), to assist in the study of sulphur compounds in oils, a CHEMIST (B.Sc.) to assist in gas adsorption studies, a CHEMICAL ENGINEER (B.Sc. or M.Sc., with a bias towards mathematics) for basic combustion research, and a CHEMICAL ENGINEER (B.Sc. or M.Sc.) to work on pipe-lining research—The Secretary, Research Council of Alberta, 87th Avenue and 114th Street, Edmonton, Alberta, Canada.

EXPERIENCED PHOTOGRAPHER FOR THE PATHOLOGICAL AND BACTERIOLOGICAL RESEARCH LABORATORY—The Administrative Officer, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford.

FISHERIES OFFICER (with a university degree in marine biology and suitable postgraduate experience) in the Bahamas, to advise the Department of Agriculture on fisheries conservation, introduce new fishing methods and enforce the fisheries laws—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD. 63/27/04/T.

GEOPHYSICIST or GEOLOGICAL ENGINEER (Ph.D. or M.Sc.), to work on groundwater hydrology; and a GEOLOGIST or GEOLOGICAL ENGINEER (M.Sc.), to work on problems of applied groundwater geology and hydrology—The Secretary, Research Council of Alberta, 87th Avenue and 114th Street, Edmonton, Alberta, Canada.

MASTER TO TEACH PHYSICS to Open Scholarship level—The Headmaster, Bolton School, Bolton, Lancs.

NATIONAL RESEARCH COUNCIL AND OTHER POSTDOCTORAL FELLOWS in all branches of chemistry, and PRE-DOCTORAL TEACHING RESEARCH FELLOWS (well qualified graduate students), for research work for the M.Sc. and Ph.D. degrees—Prof. C. A. McDowell, Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada.

PHYSICAL CHEMISTS (graduates with a good honours degree, preferably with some postgraduate experience or Ph.D. degree), to join a team working on fundamental studies on the physical properties of high polymers—The Director, Arthur D. Little Research Institute, Inveresk, Scotland.

PRINCIPAL FISHERIES RESEARCH OFFICER (with an honours degree in biology with not less than six years postgraduate research experience, preferably in fisheries science or marine biology) with the Federal Government of Nigeria, to plan and carry out research connected with the sea and inland fisheries of Nigeria, including studies in the field of population dynamics, and to supervise a small research group—The Appointments Secretary, Federal Public Service Commission, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting H.1/1.

SCIENTIFIC OFFICER, CHEMIST (with a good honours degree in chemistry, and preferably with some experience in foods and oilseeds analysis, and a knowledge of fumigant and insecticide analysis) with the West African Stored Products Research Unit, Federation of Nigeria, to undertake research into problems associated with storage of oilseeds and other food crops with particular reference to insect infestation with the object of evolving methods for reducing losses—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.153/14/05/T.

SENIOR LECTURER IN ELECTRONICS, a LECTURER IN ELECTRONICS OR MEASUREMENTS, a LECTURER IN GENERAL ELECTRICAL ENGINEERING, and an ASSISTANT LECTURER IN ELECTRONICS—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

General Register Office. The Registrar General's Quarterly Return for England and Wales. Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates; quarter ended 30th September 1959. (No. 443, 3rd Quarter, 1959.) Pp. 20. (London: H.M. Stationery Office, 1959.) 2*s*. net. [22

National Union of Journalists. Freelance Directory, 1959. Pp. i+4 (London: National Union of Journalists, 1959.) [22

Agricultural Research Institute of Northern Ireland. Thirty-second Annual Report, 1958–59. Pp. 42. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1959.) [22

Department of Scientific and Industrial Research. Mechanical Engineering Research 1958: Report of the Mechanical Engineering Research Board with the Report of the Director of Mechanical Engineering Research. Pp. iii+67+4 plates. (London: H.M. Stationery Office, 1959.) 4*s*. 6*d*. net. [22

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 103: Catch Crops. Pp. 2. (Belfast: Ministry of Agriculture, 1959.) [22

Evaluation and Presentation of Spectro-Analytical Results. By Dr. A. B. Calder. Pp. 47. (London: Hilger and Watts, Ltd., 195 5*s*. net. [22

Other Countries

National Science Foundation. American Science Manpower: Employment and other Characteristics, 1954–55. (Based on the National Register of Scientific and Technical Personnel, 1954–5 Pp. vi+48. (NSF 59–39). Scientific Manpower 1958: Papers of a Symposium on Demographic and Sociological Aspects of Scientific Manpower. Pp. v+87. (NSF 59–37). (Washington, D.C.: National Science Foundation, 1959.) [22

Union of South Africa: Department of Commerce and Industries Report No. 37: The South African Pilchard (*Sardinops ocellata*) and Maasbanker (*Trachurus trachurus*). Hydrographical Environment and the Commercial Catches, 1950–57. By M. E. L. Buys. Pp. 17 (Reprint from *Commerce and Industry*). (Pretoria: Government Printer, 1959.) [22

Report on the Administration of the Meteorological Department of the Government of India in 1953–54. Pp. 76+3 plates. (Simla: Government of India Press, 1956.) [22

Indian Forest Bulletin No. 215 (New Series): Entomology. Prophylactic Efficacy of Various Insecticides in the Protection of Freshly Felled and Converted Timbers (Planks) Against Insect Borers. By M. L. Roonwal, P. N. Chatterjee and R. S. Thapa. Pp. ii+45. (Delhi: Manager of Publications, 1959.) Rs. 4.12; 6*s*. 6*d*. [22

India. Council of Scientific and Industrial Research: Central Fuel Research Institute. Report of Director for the year 1957–58. Pp. iv+115. (Jealgora Dhanbad, Bihar: Central Fuel Research Institute, 1958.) [221

Indian Forest Bulletin No. 171 (2) (New Series): Entomology. A List of Insect Pests of Forest Plants in India and the Adjacent Countries (Arranged Alphabetically According to the Plant Genera and Species, for the Use of Forest Officers). Part 3: List of Insect Pests of Plant Genera "A" (Appendix only), "B" (*Baccaurea* to *Buxus*) and "C" (in part) (*Cadaba* to *Citrus*). By G. D. Bhasin, M. L. Roonwal and Balwant Singh. Pp. i+126. (Delhi: Manager of Publications, 1959). Rs. 4.50; 7*s*. [221

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